(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 714 024 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
30.01.2002 Bulletin 2002/05

(51) Int Cl.[7]: **G01N 21/55**, C12Q 1/28, G01N 21/35

(21) Application number: **95118553.7**

(22) Date of filing: **24.11.1995**

(54) **Method of and apparatus for determining hydrogen peroxide**

Vorrichtung und Verfahren zur Bestimmung von Wasserstoffperoxid

Procédé et dispositif pour la détermination de peroxyde d'hydrogène

(84) Designated Contracting States:
CH DE FR GB LI

(30) Priority: **25.11.1994 JP 31593394**
25.11.1994 JP 31593494

(43) Date of publication of application:
29.05.1996 Bulletin 1996/22

(73) Proprietor: **Kyoto Dai-ichi Kagaku Co., Ltd.**
Minami-ku, Kyoto 601 (JP)

(72) Inventors:
- **Yamaguchi, Yoshinori,**
  Kyoto Dai-ichi Kagaku Co Ltd
  Minami-ku, Kyoto 601 (JP)
- **Yagi, Masayuki, Kyoto Dai-ichi Kagaku Co., Ltd.**
  Minami-ku, Kyoto 601 (JP)
- **Xiaoming, Dou, Kyoto Dai-ichi Kagaku Co., Ltd.**
  Minami-ku, Kyoto 601 (JP)
- **Ashibe, Emi, Kyoto Dai-ichi Kagaku Co., Ltd.**
  Minami-ku, Kyoto 601 (JP)
- **Uenoyama, Harumi,**
  Kyoto Dai-ichi Kagaku Co., Ltd.
  Minami-ku, Kyoto 601 (JP)

(74) Representative: **Schoppe, Fritz, Dipl.-Ing.**
Schoppe, Zimmermann & Stöckeler
Patentanwälte Postfach 71 08 67
81458 München (DE)

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- **PATENT ABSTRACTS OF JAPAN vol. 009 no. 193 (E-334) ,9 August 1985 & JP-A-60 060729 (HITACHI SEISAKUSHO KK) 8 April 1985,**
- **PATENT ABSTRACTS OF JAPAN vol. 010 no. 080 (P-441) ,29 March 1986 & JP-A-60 218069 (KIYOUWA MEDETSUKUSU KK) 31 October 1985,**

EP 0 714 024 B1

Printed by Jouve, 75001 PARIS (FR)

Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a method of determining hydrogen peroxide for quality-controlling a commercially available aqueous solution containing hydrogen peroxide or another substance containing hydrogen peroxide, or in a hydrogen peroxide formation or decomposition system in chemical reaction such as enzyme reaction, and an apparatus which is employed therefor.

Description of the Background Art

[0002] In relation to determination of hydrogen peroxide in an aqueous solution, the following methods are known in the art:

(1) A method employing a hydrogen peroxide electrode.
(2) Leuco or oxidation condensation type spectrophotometry (refer to Japanese Patent Laying-Open Gazette No. 59-182361 (1984)), which is typically adapted to react hydrogen peroxide with 4-aminoantipyrin and phenol for coloring, and to measure absorption of the coloring reaction solution at 505 nm.
(3) A fluorescent method, which is adapted to react hydrogen peroxide with homovanillic acid to generate fluorescence, and to measure the fluorescence.
(4) Chemiluminescence, which is adapted to excite a substrate of luminol or lucigenin through oxidizing power of hydrogen peroxide under presence of a catalyst such as POD (peroxidase) and to detect light generated when the substrate returns from the excited state to the ground state.

[0003] The aforementioned methods (1) to (4) of determining hydrogen peroxide in aqueous solution samples have the following problems:

[0004] The method (1) is adapted to measure current change which is caused when hydrogen peroxide is electrically oxidized, and hence an influence is exerted by a reducing substance coexisting in the sample solution.

[0005] In the leuco type spectrophotometry (2), an error is readily caused by coloring of a reagent blank resulting from natural oxidation of a chromogen. In the oxidation condensation type spectrophotometry (2), on the other hand, a negative error is readily caused by a reducing substance. Further, hydrogen oxide of 2 moles is required for forming a pigment of 1 mole, and hence this method is unsuitable for determination of a component of a small quantity.

[0006] In the fluorescent method (3), sensitivity remarkably depends on the performance of an apparatus. Thus, this method is extremely influenced by a temperature and a coexistent substance.

[0007] In the chemiluminescence (4), a sufficient quantity of light emission is obtained only under alkaline conditions. The reaction rate is slow and reproducibility is insufficient. Further, light emission intensity is reduced upon coexistence of protein.

[0008] JP 60218069 discloses a method of quantitively determining the concentration of hydrogen peroxide by means of coloring the sample solution by forming a pigment from a reaction between hydrogen peroxide and a chromogen and then measuring the absorbance of the sample solution.

[0009] JP 60060729 discloses a cleaning device that continuously and automatically measures the componental concentration of a cleaning solution. To this end, an ultraviolet light beam and an infrared light beam are directed as interrupted lights onto the cleaning solution and the acoustic signals generated by absorption of the interrupted lights in the cleaning solution are detected. The strength of the acoustic signals corresponds to the concentration of hydrogen peroxide and ammonia in the cleaning solution.

SUMMARY OF THE INVENTION

[0010] An object of the present invention is to enable simple quantitative analysis of hydrogen peroxide in an aqueous solution through optical analysis means.

[0011] This object is achieved by a method according to claim 1 and an apparatus according to claim 15. Hydrogen peroxide concentration is determined through absorption of hydrogen peroxide in an aqueous solution over infrared and near infrared regions.

[0012] A sample solution is in existence in a cell having a total reflection prism at least on one surface thereof, a measuring beam including light in the infrared region is introduced into the total reflection prism to be totally reflected, and absorbance at any one absorption peak which is present at 1200 to 1500 cm^{-1} or 2600 to 3000 cm^{-1} in absorption of the measuring beam caused in the interface between the total reflection prism and the sample solution is measured, thereby determining hydrogen peroxide. The absorbance in total reflection is measured from intensity of attenuated total reflection

[0013] A hydrogen peroxide determination apparatus making measurement in the infrared region comprises a total reflection cell having a total reflection prism consisting of a material having a larger refractive index than a sample solution on at least one of wall surfaces defining a space storing the sample solution, an incident optical system, including an infrared light source, for introducing a measuring beam of the infrared region into the total reflection prism at an angle of incidence causing total reflection, and a measuring optical system receiving an outgoing beam from the total reflection prism for measuring absorbance of at least one absorption peak

which is present at 1200 to 1500 cm^{-1} or 2600 to 3000 cm^{-1}.

[0014] The light source which is included in the incident optical system is that generating a beam of a continuous wavelength, and a spectroscope is included in the incident optical system or the measuring optical system to make spectral measurement. The measuring beam includes a continuous wavelength beam of a mid infrared region including 1200 to 1500 cm^{-1} and 2600 to 3000 cm^{-1}.

[0015] Fig. 1 schematically illustrates a method employing a total reflection cell 2. At least one surface of the total reflection cell 2 is formed by a total internal reflection prism 4. A sample solution 8 is in existence in the total reflection cell 2 and a measuring beam is introduced into the total reflection prism 4 from an illuminating optical system 16 at an angle θ of incidence for causing total reflection, whereby the measuring beam is transmitted through the total reflection prism 4 while being totally reflected. At this time, the measuring beam slightly permeates toward the sample solution 8 in the interface between the total internal reflection prism 4 and the sample solution 8, and a specific wavelength component of an evanescent wave of excitation energy of the measuring beam is absorbed by hydrogen peroxide. The outgoing beam from the total internal reflection prism 4 is separated into its spectral components by a measuring optical system 18 so that absorbance by total reflection of a characteristic wavelength component thereof is measured, thereby determining hydrogen peroxide in the sample solution.

[0016] Assuming that n_2 represents the refractive index of a sample solution and n_1 ($n_2 < n_1$) represents that of a total refection prism, a critical angle θc causing total reflection is expressed as follows:

$$\theta c = \sin^{-1}(n_2/n_1) \qquad (\text{where } 0° < \theta c < 90°)$$

The angle θ of incidence upon the interface between the total reflection prism 4 and the sample solution 8 in the case of introducing a measuring beam into the total reflection prism 4 from the incident optical system 16 is set in the following condition:

$$\theta > \theta c$$

[0017] Absorption in the total reflection prism 4 is expressed by absorbance A as follows:

$A = N \cdot \alpha \cdot de \cdot \log e$ where N represents the number of times of total reflection in the total reflection prism 4, α represents the absorption coefficient of the sample solution 8, and de represents the optical path length along which the measuring beam permeates into the sample solution 8 in single total reflection.

[0018] In a method employing absorption in the near infrared region, on the other hand, a measuring beam including light of the near infrared region is introduced into a sample solution which is in existence in a light-transmittable cell, and hydrogen peroxide is determined on the basis of absorbance of any absorption peak which is present at 4300 to 4800 cm^{-1} or 5400 to 6600 cm^{-1} in the transmitted light.

[0019] A hydrogen peroxide determination/measuring apparatus which is applied for making measurement in the near infrared region employs a light-transmittable cell, in place of the total reflection cell in the measuring apparatus for the first method.

[0020] The method according to the present invention can be applied not only to measurement of an aqueous solution sample already containing hydrogen peroxide, but to monitoring of a reaction system such as enzyme reaction of forming or decomposing hydrogen peroxide.

[0021] In a first example, the method according to the present invention is applied to a reaction system of forming hydrogen peroxide by specific reaction between an oxidizing enzyme and a biological or metabolic component. Assuming that S represents a substrate, P represents a product and E represents an enzyme, the quantity of hydrogen peroxide which is formed by the following reaction is measured by the method according to the present invention. The quantity of the substrate S or the product P can be obtained from the measured quantity of hydrogen peroxide. Further, enzyme activity can also be measured from the measured quantity of hydrogen peroxide.

$$S + O_2 \xrightarrow{E} P + H_2O_2$$

[0022] Exemplary combinations of the substrate S and the enzyme E are glucose and glucose oxidase, cholesterol and cholesterol oxidase, urea and uricase, pyruvic acid and oxidase pyruvate, and hexose and pyranose oxidase, while the combination is not restricted to these so far as enzyme reaction of forming hydrogen peroxide is caused.

[0023] A second example is adapted to make reaction through an enzyme which is specifically reacted with hydrogen peroxide and decomposes the same, for measuring hydrogen peroxide by the method according to the present invention. Assuming that PH_2 represents a reactant, P represents a product and E represents an enzyme, the quantity of the reactant PH_2 or the product P can be obtained by measuring the quantity of reduced hydrogen peroxide by the following enzyme reaction. Further, enzyme activity can also be measured by the measured quantity of reduced hydrogen peroxide.

$$H_2O_2 + PH_2 \xrightarrow{E} P + 2H_2O$$

[0024] While the enzyme can be a dehydrogenase such as peroxidase or catalase, the present invention is also applicable to reaction which is related to another enzyme so far as the same is conjugate reaction with hydrogen peroxide.

[0025] It is possible to first label a reactant with a compound such as peroxidase or catalase having reactivity with hydrogen peroxide and to thereafter react the reactant with a constant quantity of hydrogen peroxide, thereby estimating the quantity of the reactant from that of reduced hydrogen peroxide. For example, the quantity of an antibody is measured by reacting an anti-antibody, which is labelled with peroxidase, with an antigen-antibody reaction combination, performing BF separation of separating and removing the unreacted labelled anti-antibody from that reacted with the antigen-antibody reaction combination, and thereafter reacting peroxidase with hydrogen peroxide, for measuring the quantity of reduced hydrogen peroxide. Either the antigen or the antibody, or either the antibody or the anti-antibody may be labelled. The antigen-antibody reaction is well known to those skilled in the art, and a method of making the antigen-antibody reaction is not limited.

[0026] Most enzyme reaction generates hydrogen peroxide. While enzyme reaction is generally monitored with a color former, the enzyme reaction can be directly monitored by the inventive method with no employment of a color former.

[0027] The foregoing and other objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of the present invention when taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028]

Fig. 1 schematically illustrates a first method of the present invention employing a total reflection cell;
Fig. 2 is a block diagram schematically showing a measuring apparatus according to an embodiment of the present invention;
Fig. 3A is a front sectional view showing a first exemplary total reflection cell having only one opening;
Fig. 3B shows front and top sectional views of a second exemplary total reflection cell having only one opening;
Fig. 4A shows perspective and front sectional views of a throwaway total reflection cell respectively, and Figs. 4B to 4E show perspective and front sectional views of exemplary total reflection flow cells respectively;
Fig. 5 illustrates total reflection absorption spectra of hydrogen peroxide standard samples of some concentration levels and distilled water;
Fig. 6 illustrates a hydrogen peroxide calibration curve based on absorbance values at the peak position of an absorption wavenumber of 1388.2 cm^{-1} in the total reflection absorption spectra shown in Fig. 5;
Fig. 7 illustrates a hydrogen peroxide calibration curve based on absorbance values at the peak position of an absorption wavenumber of 2831.9 cm^{-1} in the total reflection absorption spectra shown in Fig. 5;
Fig. 8 illustrates total reflection absorption spectra of a commercially available contact lens washing solution and distilled water;
Fig. 9 illustrates the absorbance values at the peak position of 1388.2 cm^{-1} in the total reflection absorption spectra shown in Fig. 8, which are applied to the calibration curve of Fig. 6;
Fig. 10 illustrates the absorbance values at the peak position of 2831.9 cm^{-1} in the total reflection absorption spectra shown in Fig. 8, which are applied to the calibration curve of Fig. 7;
Fig. 11 illustrates total reflection absorption spectra of a commercially available peroxide antiseptic solution and distilled water;
Fig. 12 illustrates the absorbance values at the peak position of 1388.2 cm^{-1} in the total reflection absorption spectra shown in Fig. 11, which are applied to the calibration curve of Fig. 6;
Fig. 13 illustrates the absorbance values at the peak position of 2831.9 cm^{-1} in the total reflection absorption spectra shown in Fig. 11, which are applied to the calibration curve of Fig. 7;
Fig. 14 illustrates near infrared absorption spectra of hydrogen peroxide standard samples of 10 to 30 % and distilled water;
Fig. 15 illustrates near infrared absorption spectra of hydrogen peroxide standard samples of 0.3 to 2.5 % and distilled water;
Fig. 16 shows a hydrogen peroxide calibration curve of the absorption spectra shown in Figs. 14 and 15 at an absorption wavenumber of 4700 cm^{-1};
Fig. 17 shows a hydrogen peroxide calibration curve of the absorption spectra shown in Figs. 14 and 15 at an absorption wavenumber of 5550 cm^{-1};
Fig. 18 shows a hydrogen peroxide calibration curve of the absorption spectra shown in Figs. 14 and 15 at an absorption wavenumber of 5860 cm^{-1};
Fig. 19 shows a hydrogen peroxide calibration curve of the absorption spectra shown in Figs. 14 and 15 at an absorption wavenumber of 6300 cm^{-1};
Fig. 20 illustrates a near infrared absorption spectrum of a commercially available peroxide antiseptic solution; and
Fig. 21 illustrates a near infrared absorption spectrum of a commercially available contact lens washing solution.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0029] Fig. 2 schematically illustrates a measuring apparatus according to an embodiment of the present invention. A measuring cell 102 is formed by a total reflection cell for measuring absorption in the infrared region, or by a light-transmittable cell for measuring absorption in the near infrared region.

[0030] The total reflection cell has a total reflection prism on at least one surface thereof. This total reflection cell can be formed by a cell having only one opening for receiving a sample solution, or a flow cell, having an inlet port and an outlet port, which is fed with the sample solution. The material for the total internal reflection prism can be prepared from ZnSe, Ge, Si, Al_2O_3 or MgO. This material may be employed for only the total reflection cell, or for all wall surfaces of the total reflection cell including the total internal reflection prism.

[0031] On the other hand, the light-transmittable cell is made of a material such as glass, quartz or polyethylene terephthalate, for receiving or passing the sample solution.

[0032] An incident optical system 16 includes an infrared light source 16a generating a measuring beam and a beam adjusting optical system 16b. The light source 16a generates a beam including a continuous wavelength beam. A fluorescent lamp, a xenon lamp or a black body radiation source can be employed for infrared light. On the other hand, a halogen lamp, a fluorescent lamp, a xenon lamp or a black-body radiation source can be employed for near infrared light. That generating a wide wavelength beam over the infrared and near infrared regions can be applied to measurement in both regions in common.

[0033] The beam adjusting optical system 16b includes an optical system for converting the beam from the light source 16a into a parallel beam, a beam splitter for separating the same into a measuring beam 20s and a reference beam 20r, and an optical system for introducing the measuring beam 20s into the measuring cell 102. In the case that the measuring cell 102 is formed by a total reflection cell, the beam adjusting optical system 16b is so adjusted as to introduce the beam into the total reflection prism at an angle of incidence causing total reflection.

[0034] An optical system 22 for adjusting a luminous flux of the measuring beam 20s totally reflected by and transmitted through the total reflection prism or that transmitted through the light-transmittable cell, and a spectroscope 23 such as an FTIR (Fourier transformation infrared spectrophotometer) for receiving the measuring beam 20s which is adjusted by the optical system 22 and separating the measuring beam 20s into its spectral parts are arranged on an optical path of the measuring beam 20s, so that the measuring beam 20s which is separated into its spectral parts is guided to and detected by a detection part 26. The measuring optical system 18 in Fig. 1 comprises the optical system 22, the spectroscope 23 and the detection part 26 in Fig. 2.

[0035] On the other hand, an optical system 24 for adjusting the luminous flux of the reference beam 20r is arranged on the optical path of the reference beam 20r for correcting fluctuation of the measuring beam 20s, so that the adjusted reference beam 20r is guided to and detected by the detection part 26. The detection part 26 is adapted to correct the measuring beam 20s which is separated into its spectral parts through the measuring cell 102 arid the spectroscope 23 with intensity of the reference beam 20r indicating light source intensity for calculating absorbance.

[0036] Numeral 28 denotes a controller which controls the spectral operation at the spectroscope 23 and transmits a detection output of the detection part 26 to a data processing unit 30. Numeral 32 denotes an output unit such as a recorder or a CRT outputting a processing result of the data processing unit 30.

[0037] Figs. 3A to 4E illustrate exemplary total reflection cells.

[0038] Figs. 3A and 3B illustrate exemplary reflection cells having only single openings. Fig. 3A shows the cell 2 illustrated in Fig. 1, having a total reflection prism 4 on its bottom surface. Fig. 3B shows front and top sectional views of a cell 40 having a total reflection prism 4 on its side surface respectively. A measuring beam 20s is introduced into the prism 4 in a horizontal plane.

[0039] Figs. 4A to 4E show perspective and front sectional views of cells having other shapes respectively.

[0040] Fig. 4A shows a single-sided throwaway cell, which is provided with a narrow clearance 42 for sucking a sample solution by a capillary phenomenon, and a total internal reflection prism 4 is formed along the clearance 42.

Numeral 15 denotes the sample solution which is sucked along the clearance 42.

[0041] Fig. 4B shows an exemplary double-sided total reflection flow cell, which is provided with total internal reflection prisms 4 and 4' on upper and lower surfaces thereof through a space fed with a sample solution. Numeral 44 denotes an inlet port for introducing the sample solution into the cell, and numeral 46 denotes an outlet port for the sample solution.

[0042] Fig. 4C shows an exemplary cylindrical surface total reflection flow cell, which is formed to enclose a side surface of a cylindrical total reflection prism 4, so that a sample solution 15 is fed along the cylindrical surface of the total reflection prism 4.

[0043] Fig. 4D shows another exemplary double-sided total reflection flow cell, which is so formed that a sample solution 15 flows along two opposite planes of a total reflection prism 4 respectively.

[0044] Fig. 4E shows an exemplary single-sided total reflection flow cell, which has a total reflection prism 4 on one surface defining a space fed with a sample solution 15.

(Example 1)

[0045] Example of making measurement with a total reflection cell comprising a total reflection prism of a ZnSe crystal is now described.

(Formation of Calibration Curve)

[0046] A standard hydrogen peroxide reagent of 30 % (Lot 3018930428 by Santoku Chemical Industries Co., Ltd.) was diluted with distilled water to prepare hydrogen peroxide standard samples of 10 %, 5 %, 3 % and 1 % respectively, and total reflection absorption spectra of these standard samples and water were measured. Fig. 5 shows the results. The spectrum of the standard sample of 1 % is omitted since the same is approximate to that of the distilled water. Absorption peaks of hydrogen peroxide are observed at positions of wavenumbers 1388.2 cm^{-1} and 2831.9 cm^{-1} respectively.

[0047] Fig. 6 shows a hydrogen peroxide calibration curve formed by plotting the absorbance values at the peak position of 1388.2 cm^{-1} in the spectra shown in Fig. 5 on the axis of ordinates while plotting concentration values on the axis of abscissas.

[0048] Fig. 7 shows a hydrogen peroxide calibration curve formed by plotting the absorbance values at the peak position of 2831.9 cm^{-1} in the spectra shown in Fig. 5 on the axis of ordinates while plotting concentration values on the axis of abscissas.

(Sample Measurement)

[0049] Examples of determining commercially available hydrogen peroxide solutions with these calibration curves are now described.

(1) Measurement of Commercially Available Contact Lens Washing Solution:

[0050] Similarly to the measurement for formation of the calibration curves, a commercially available contact lens washing solution (Consent F (trade name) imported by Barndshaind Co., Ltd.) (calculated as 2.98 % from indicated concentration) was measured to obtain a total reflection absorption spectrum ① in Fig. 8. ② shows a total reflection absorption spectrum of distilled water which was measured in a similar manner.

[0051] Absorbance of the spectrum at a peak position of 1388. 2 cm^{-1} was calculated and applied to the calibration curve of Fig. 6, thereby estimating hydrogen peroxide concentration of 3.11 %, as shown in Fig. 9.

[0052] Absorbance of the spectrum at the peak position of 2831.9 cm^{-1} was calculated and applied to the calibration curve of Fig. 7, thereby estimating hydrogen peroxide concentration of 3.18 %, as shown in Fig. 10.

(2) Measurement of Commercially Available Peroxide Antiseptic Solution:

[0053] Similarly to the measurement for formation of the calibration curves, a commercially available peroxide antiseptic solution (product by Fujimi Seiyaku Co., Ltd. ; indicated as 3 W/V %) was measured to obtain a total reflection absorption spectrum ① shown in Fig. 11. ② shows a total reflection absorption spectrum of distilled water which was measured in a similar manner.

[0054] Absorbance of the spectrum at the peak position of 1388.2 cm^{-1} was calculated and applied to the calibration curve of Fig. 6, thereby estimating hydrogen peroxide concentration of 3.26 %, as shown in Fig. 12.

[0055] Absorbance of the spectrum at the peak position of 2831.9 cm^{-1} was calculated and applied to the calibration curve of Fig. 7, thereby estimating hydrogen peroxide concentration of 3.32 %, as shown in Fig. 13.

[0056] Thus, hydrogen peroxide can be determined through absorbance of a total reflection absorption spectrum of a sample solution containing hydrogen peroxide at the peak position of 1388.2 cm^{-1} or 2831.9 cm^{-1}.

(Example 2)

[0057] Example of making measurement by employing a light-transmittable cell which was made of a quartz crystal, a halogen lamp as a light source and FTIR (Perkin Elmer System 2000) as a spectroscope is now described.

(Formation of Calibration Curve)

[0058] The same standard hydrogen peroxide reagent of 30 % (W/V) as that in Example 1 was diluted with distilled water to prepare hydrogen peroxide standard samples of 30 %, 20 %, 10 %, 2.5 %, 1.5 %, 0.75 %, 0.3 %, 0.225 %, 0. 15 %, 0.075 % and 0.03 % respectively, and absorption spectra of these standard samples and distilled water were measured. Figs. 14 and 15 show some of the results in high and low concentration regions respectively. The absorption spectrum of distilled water was subtracted from those of the standard samples in Fig. 14 and 15, and hence absorbance values of the hydrogen peroxide absorption peaks appear on positive sides. The absorption peaks are recognized in the vicinity of 4300 to 4800 cm^{-1}, 5500 cm^{-1}, 5860 cm^{-1} and 6300 cm^{-1} as characteristic peaks of hydrogen peroxide.

[0059] Figs. 16 to 19 illustrate hydrogen peroxide calibration curves which are formed by plotting absorbance values at peak positions of respective absorption wavenumbers on the axis of ordinates while plotting concentration values on the axis of abscissas and applying straight lines thereto by least square fitting.

(Sample Measurement)

[0060] Examples of determining commercially availa-

ble hydrogen peroxide solutions with these calibration curves are now described.

(1) Measurement of Commercially Available Peroxide Antiseptic Solution:

[0061] Similarly to the measurement for formation of the calibration curves, the same commercially available peroxide antiseptic solution as that in Example 1 was measured, to obtain an absorption spectrum shown in Fig. 20. Absorbance values at respective absorption peaks in the vicinity of 4700 cm^{-1}, 5500 cm^{-1}, 5860 cm^{-1} and 6300 cm^{-1} in this spectrum were obtained and the respective calibration curves shown in Figs. 16 to 19 were applied to these absorbance values, thereby estimating hydrogen peroxide concentration values of 3.26 %, 3.21 %, 3.31 % and 3.29 % respectively.

(2) Measurement of Commercially Available Contact Lens Washing Solution:

[0062] Similarly to the measurement for formation of the calibration curves, the same commercially available contact lens washing solution as that in Example 1 was measured, to obtain an absorption spectrum shown in Fig. 21. Absorbance values at respective absorption peaks in the vicinity of 4300 to 4800 cm^{-1}, 5500 cm^{-1}, 5860 cm^{-1} and 6300 cm^{-1} in this spectrum were obtained and the respective calibration curves shown in Figs. 16 to 19 were applied to these absorbance values, thereby estimating hydrogen oxide concentration values of 3.12 %, 3.22 %, 3. 18 % and 3.08 % respectively.

[0063] Thus, it is possible to determine hydrogen peroxide through any absorption peak, which is present at 4300 to 4800 cm^{-1} or 5400 to 6600 cm^{-1}, of an infrared absorption spectrum of a sample solution containing hydrogen peroxide.

Claims

1. A method of determining concentration of hydrogen peroxide, comprising the following steps:

   introducing any wavelength beam over near infrared to infrared wavelength regions into an aqueous solution sample, and

   measuring light absorption of hydrogen peroxide, thereby determining the concentration of hydrogen peroxide on the basis of absorbance at any absorption peak, wherein

   said aqueous solution sample (8) is in existence in a measuring cell (2) having a total reflection prism (4) on at least one surface thereof, or in a light-transmittable cell, and

   a measuring beam of said infrared region is introduced into said total reflection prism (4) or into the said aqueous sample solution being in existence in the light-transmittable cell to be totally reflected.

2. The determination method in accordance with claim 1, wherein said aqueous solution sample (8) is in existence in a measuring cell (2) having a total reflection prism (4) on at least one surface thereof and the determination of said hydrogen peroxide is performed on the basis of absorbance of any absorption peak being present at 1200 to 1500 cm^{-1} or 2600 to 3000 cm^{-1} in absorption of said measuring beam caused in an interface between said total reflection prism (4) and said aqueous solution sample (8).

3. The determination method in accordance with claim 1, wherein said aqueous solution sample (8) is in existence in a light-transmittable cell and the determination of said hydrogen peroxide is performed on the basis of absorbance of any absorption peak being present at 4300 to 4800 cm^{-1} or 5400 to 6600 cm^{-1} in light being transmitted therethrough.

4. The determination method in accordance with claim 1, wherein said aqueous solution sample already contains hydrogen peroxide.

5. The determination method in accordance with claim 1, wherein said aqueous solution sample is a reactive solution being so prepared as to cause enzyme reaction of generating hydrogen peroxide, and a reaction system generating hydrogen peroxide by the following enzyme reaction assuming that S represents a substrate, P represents a product and E represents an enzyme:

$$S + O_2 \xrightarrow{E} P + H_2O_2$$

6. The determination method in accordance with claim 5, wherein the quantity of said substrate S or said product P is obtained by measurement of the quantity of generated hydrogen peroxide.

7. The determination method in accordance with claim 5, wherein enzyme activity is measured by measuring the quantity of generated hydrogen peroxide.

8. The determination method in accordance with claim 5, wherein said enzyme is an oxidase.

9. The determination method in accordance with claim 8, wherein a combination of said substrate and said

enzyme is selected from a group consisting of glucose and glucose oxidase, cholesterol and cholesterol oxidase, urea and uricase, pyruvic acid and oxidase pyruvate, and hexose and pyranose oxidase.

10. The determination method in accordance with claim 1, wherein said aqueous solution sample is a reactive solution being so prepared as to cause enzyme reaction of decomposing hydrogen peroxide, and a reaction system reducing hydrogen peroxide in the following enzyme reaction assuming that PH2 represents a react ant, P represents a product and E represents an enzyme:

$$H_2O_2 + PH_2 \xrightarrow{E} P + 2H_2O$$

11. The determination method in accordance with claim 10, wherein the quantity of said react ant PH_2 or said product P is obtained by measuring the amount of reduced hydrogen peroxide.

12. The determination method in accordance with claim 10, wherein said enzyme E is a dehydrogenase consisting of peroxidase or catalase.

13. The determination method in accordance with claim 1, wherein said aqueous solution sample is a reaction system containing a reactant labelled with a compound having reactivity with hydrogen peroxide and a constant quantity of hydrogen peroxide, the quantity of said react ant being obtained from that of reduced hydrogen peroxide.

14. The determination method in accordance with claim 13, wherein an anti-antibody being labelled with peroxidase is reacted with an antigen-antibody reaction combination, subjected to BF separation and thereafter reacted with hydrogen peroxide so that the quantity of said antibody is obtained by measuring that of reduced hydrogen peroxide.

15. A hydrogen peroxide concentration determination apparatus comprising:

a measuring cell (102) defining a space for receiving a sample solution;

an illuminating optical system (16) for introducing a measuring beam of an infrared region into said measuring cell (102); and

a measuring optical system (18) for receiving an outgoing beam from said measuring cell (102) for measuring absorbance of at least one absorption peak,

characterized in that

the measuring cell (102) is formed by a total reflection cell (2, 40) having a total reflection prism (4) on at least one of wall surfaces;

the illuminating optical system (16) is adapted to introduce the measuring beam into the total reflection prism (4) at an angle of incidence for causing total reflection; and

the at least one absorption peak is present at 1200 to 1500 cm^{-1} or 2600 to 3000 cm^{-1}.

16. The hydrogen peroxide concentration determination apparatus in accordance with claim 15, wherein said total reflection cell (2, 40) is a cell having only one opening.

17. The hydrogen peroxide concentration determination apparatus in accordance with claim 15, wherein said total reflection cell is a flow cell, having an inlet port and an outlet port, for being fed with said sample solution.

Patentansprüche

1. Ein Verfahren zum Bestimmen einer Konzentration von Wasserstoffperoxid, das folgende Schritte aufweist:

Einbringen eines Strahls beliebiger Wellenlänge über Nahe-Infrarot- bis zu Infrarot-Wellenlängen-Bereiche in eine wäßrige Lösungsprobe, und

Messen einer Lichtabsorption von Wasserstoffperoxid, wodurch die Konzentration an Wasserstoffperoxid auf der Basis einer Absorbanz bei einer beliebigen Absorptionsspitze bestimmt wird, wobei

die wäßrige Lösungsprobe (8) in einer Meßzelle (2), die an mindestens einer Oberfläche derselben ein Totalreflexionsprisma (4) aufweist, oder in einer lichtdurchlässigen Zelle vorliegt, und

ein Meßstrahl des Infrarotbereichs in das Totalreflexionsprisma (4) oder in die wäßrige Probenlösung, die in der lichtdurchlässigen Zelle vorliegt, eingebracht wird, um vollständig reflektiert zu werden.

2. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe (8) in einer Meßzelle (2), die an mindestens einer Oberfläche

derselben ein Totalreflexionsprisma (4) aufweist, vorliegt, und bei dem die Bestimmung des Wasserstoffperoxids auf der Basis einer Absorbanz einer beliebigen Absorptionsspitze, die bei 1200 bis 1500 cm^{-1} oder 2600 bis 3000 cm^{-1} vorliegt, bei einer Absorption des Meßstrahls, die in einer Schnittstelle zwischen dem Totalreflexionsprisma (4) und der wäßrigen Lösungsprobe (8) bewirkt wird, durchgeführt wird.

3. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe (8) in einer lichtdurchlässigen Zelle vorliegt und die Bestimmung des Wasserstoffperoxids auf der Basis einer Absorbanz einer beliebigen Absorptionsspitze, die bei 4300 bis 4800 cm^{-1} oder 5400 bis 6600 cm^{-1} vorliegt, in Licht, das durch dieselbe durchgelassen wird, durchgeführt wird.

4. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe bereits Wasserstoffperoxid enthält.

5. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe eine reaktive Lösung ist, die präpariert ist, um eine Enzymreaktion des Erzeugens von Wasserstoffperoxid und ein Reaktionssystem zu bewirken, das durch die folgende Enzymreaktion unter der Annahme, daß S ein Substrat darstellt, P ein Produkt darstellt und E ein Enzym darstellt, Wasserstoffperoxid erzeugt:

$$S + O_2 \xrightarrow{E} P + H_2O_2$$

6. Das Bestimmungsverfahren gemäß Anspruch 5, bei dem die Menge des Substrats S oder des Produkts P durch Messung der Menge an erzeugtem Wasserstoffperoxid erhalten wird.

7. Das Bestimmungsverfahren gemäß Anspruch 5, bei dem eine Enzymaktivität durch Messen der Menge an erzeugtem Wasserstoffperoxid gemessen wird.

8. Das Bestimmungsverfahren gemäß Anspruch 5, bei dem das Enzym eine Oxidase ist.

9. Das Bestimmungsverfahren gemäß Anspruch 8, bei dem eine Kombination des Substrats und des Enzyms aus einer Gruppe ausgewählt wird, die aus Glukose und Glukoseoxidase, Cholesterol und Cholesteroloxidase, Harnstoff und Uricase, Pyruvinsäure und Oxidasepyruvat sowie Hexose und Pyranoseoxidase besteht.

10. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe eine reaktive Lösung ist, die präpariert ist, um eine Enzymreaktion des Zerlegens von Wasserstoffperoxid und ein Reaktionssystem zu bewirken, das in der folgenden Enzymreaktion unter der Annahme, daß PH_2 einen Reaktionspartner darstellt, P ein Produkt darstellt und E ein Enzym darstellt, Wasserstoffperoxid reduziert:

$$H_2O_2 + PH_2 \xrightarrow{E} P + 2H_2O$$

11. Das Bestimmungsverfahren gemäß Anspruch 10, bei dem die Menge des Reaktionspartners PH_2 oder des Produkts P durch Messen der Menge an reduziertem Wasserstoffperoxid erhalten wird.

12. Das Bestimmungsverfahren gemäß Anspruch 10, bei dem das Enzym E eine aus Peroxidase oder Katalase bestehende Dehydrogenase ist.

13. Das Bestimmungsverfahren gemäß Anspruch 1, bei dem die wäßrige Lösungsprobe ein Reaktionssystem ist, das einen Reaktionspartner, der mit einer Verbindung markiert ist, welche eine Reaktionsbereitschaft gegenüber Wasserstoffperoxid aufweist, sowie eine konstante Menge an Wasserstoffperoxid enthält, wobei die Menge des Reaktionspartners von der an reduziertem Wasserstoffperoxid erhalten wird.

14. Das Bestimmungsverfahren gemäß Anspruch 13, bei dem ein mit Peroxidase markierter Anti-Antikörper mit einer Antigen-Antikörper-Reaktionskombination zur Reaktion gebracht, einer BF-Trennung unterworfen und daraufhin mit Wasserstoffperoxid zur Reaktion gebracht wird, so daß die Menge des Antikörpers durch Messen derjenigen an reduziertem Wasserstoffperoxid erhalten wird.

15. Eine Vorrichtung zur Bestimmung einer Wasserstoffperoxidkonzentration, welche folgende Merkmale aufweist:

eine Meßzelle (102), die einen Raum zum Aufnehmen einer Probenlösung definiert;

ein optisches Beleuchtungssystem (16) zum Einbringen eines Meßstrahls eines Infrarotbereichs in die Meßzelle (102); und

ein optisches Meßsystem (18) zum Aufnehmen eines von der Meßzelle (102) ausgehenden Strahls zum Messen einer Absorbanz von mindestens einer Absorptionsspitze,

dadurch gekennzeichnet, daß

die Meßzelle (102) durch eine Totalreflexionszelle (2, 40) gebildet ist, die an mindestens einer von Wandoberflächen ein Totalreflexionsprisma (4) aufweist;

das optische Beleuchtungssystem (16) ausgelegt ist, den Meßstrahl bei einem Einfallswinkel zum Bewirken einer Totalreflexion in das Totalreflexionsprisma (4) einzubringen; und

die mindestens eine Absorptionsspitze bei 1200 bis 1500 cm^{-1} oder 2600 bis 3000 cm^{-1} vorliegt.

16. Die Vorrichtung zur Bestimmung einer Wasserstoffperoxidkonzentration gemäß Anspruch 15, bei der die Totalreflexionszelle (2, 40) eine Zelle ist, die lediglich eine Öffnung aufweist.

17. Die Vorrichtung zur Bestimmung einer Wasserstoffperoxidkonzentration gemäß Anspruch 15, bei der die Totalreflexionszelle eine Durchflußzelle ist, die eine Einlaßöffnung und eine Auslaßöffnung zum Zuführen der Probenlösung zu derselben aufweist.

Revendications

1. Procédé pour déterminer la concentration de peroxyde d'hydrogène, comprenant les étapes suivantes consistant à :

   - introduire un faisceau de toute longueur d'onde dans les régions presque infrarouge à infrarouge dans un échantillon de solution aqueuse, et
   - mesurer l'absorption de lumière du peroxyde d'hydrogène, déterminant ainsi la concentration de peroxyde d'hydrogène sur base de l'absorbance à toute pointe d'absorption, dans lequel
   - ledit échantillon de solution aqueuse (8) est présent dans une cellule de mesure (2) ayant un prisme à réflexion totale (4) sur au moins une surface de celle-ci, ou dans une cellule à capacité de transmission de lumière, et
   - un faisceau de mesure de ladite région infrarouge est introduit dans ledit prisme à réflexion totale (4) ou dans ledit échantillon de solution aqueuse présent dans la cellule à capacité de transmission de lumière, pour être totalement réfléchi.

2. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse (8) est présent dans une cellule de mesure (2) ayant un prisme à réflexion total (4) sur au moins une surface de celle-ci et la détermination dudit peroxyde d'hydrogène s'effectue sur base de l'absorbance de toute pointe d'absorption présente à de 1200 à 1500 cm^{-1} ou de 2600 à 3000 cm^{-1} dans l'absorption dudit faisceau de mesure provoquée dans une interface entre ledit prisme à réflexion totale (4) et ledit échantillon de solution aqueuse (8).

3. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse (8) est présent dans une cellule à capacité de transmission de lumière et la détermination dudit peroxyde d'hydrogène s'effectue sur base de l'absorbance de toute pointe d'absorption présente à de 4300 à 4800 cm^{-1} ou de 5400 à 6600 cm^{-1} dans la lumière transmise à travers celle-ci.

4. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse contient déjà le peroxyde d'hydrogène.

5. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse est une solution réactive préparée de manière à provoquer une réaction enzymatique générant du peroxyde d'hydrogène et un système de réaction générant du peroxyde d'hydrogène par la réaction enzymatique suivante, en supposant que S représente un substrat, P représente un produit et E représente une enzyme :

$$S + O_2 \xrightarrow{E} P + H_2O_2.$$

6. Procédé de détermination suivant la revendication 5, dans lequel la quantité dudit substrat S ou dudit produit P est obtenue en mesurant la quantité de peroxyde d'hydrogène générée.

7. Procédé de détermination suivant la revendication 5, dans lequel l'activité enzymatique est mesurée en mesurant la quantité de peroxyde d'hydrogène générée.

8. Procédé de détermination suivant la revendication 5, dans lequel ladite enzyme est une oxydase.

9. Procédé de détermination suivant la revendication 8, dans lequel une combinaison dudit substrat et de ladite enzyme est choisie parmi un groupe composé de glucose et glucose-oxydase, de cholestérol et de cholestérol-oxydase, d'urée et d'uricase, d'acide pyruvique et de pyruvate d'oxydase, et d'hexose et de pyranoseoxydase.

10. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse est une solution réactive préparée de manière à

provoquer une réaction enzymatique de décomposition de peroxyde d'hydrogène dans la réaction enzymatique suivante, en supposant que PH_2 représente un réactif, P représente un produit et E représente une enzyme :

$$H_2O_2 + PH_2 \xrightarrow{E} P + 2H_2O.$$

11. Procédé de détermination suivant la revendication 10, dans lequel la quantité dudit réactif PH_2 ou dudit produit P est obtenue en mesurant la quantité de peroxyde d'hydrogène réduit.

12. Procédé de détermination suivant la revendication 10, dans lequel ladite enzyme E est une déshydrogénase consistant en peroxydase ou catalase.

13. Procédé de détermination suivant la revendication 1, dans lequel ledit échantillon de solution aqueuse est un système de réaction contenant un réactif marqué à l'aide d'un composé ayant une réactivité avec le peroxyde d'hydrogène et une quantité constante de peroxyde d'hydrogène, la quantité dudit réactif étant obtenue à partir de celle du peroxyde d'hydrogène réduit.

14. Procédé de détermination suivant la revendication 13, dans lequel un anti-anticorps marqué à l'aide de peroxydase est réagi par une combinaison de réaction antigène-anticorps, soumis à une séparation BF et, ensuite, réagi avec du peroxyde d'hydrogène, de sorte que la quantité dudit anticorps est obtenue en mesurant celle du peroxyde d'hydrogène réduit.

15. Appareil pour déterminer la concentration de peroxyde d'hydrogène, comprenant :

- une cellule de mesure (102) définissant un espace destiné à recevoir un échantillon de solution ;
- un système optique d'illumination (16) destiné à introduire un faisceau de mesure d'une région infrarouge dans ladite cellule de mesure (102) ; et
- un système optique de mesure (18) destiné à recevoir un faisceau sortant de ladite cellule de mesure (102), pour mesurer l'absorbance d'au moins une pointe d'absorption,

caractérisé par le fait que :

- la cellule de mesure (102) est formée par une cellule à réflexion totale (2, 40) ayant un prisme à réflexion totale (4) sur au moins l'une des surfaces de paroi ;
- le système optique d'illumination (16) est adapté pour introduire le faisceau de mesure dans le prisme à réflexion totale (4) suivant un angle d'incidence tel qu'il entraîne une réflexion totale ; et
- au moins une pointe d'absorption est présente à de 1200 à 1500 cm^{-1} ou de 2600 à 3000 cm^{-1}.

16. Appareil pour déterminer la concentration de peroxyde d'hydrogène suivant la revendication 15, dans lequel ladite cellule à réflexion totale (2, 40) est une cellule ne présentant qu'une seule ouverture.

17. Appareil pour déterminer la concentration de peroxyde d'hydrogène suivant la revendication 15, dans lequel ladite cellule à réflexion totale est une cellule d'écoulement présentant un orifice d'entrée et un orifice de sortie, destinée à être alimentée par ledit échantillon de solution.

Fig. 1

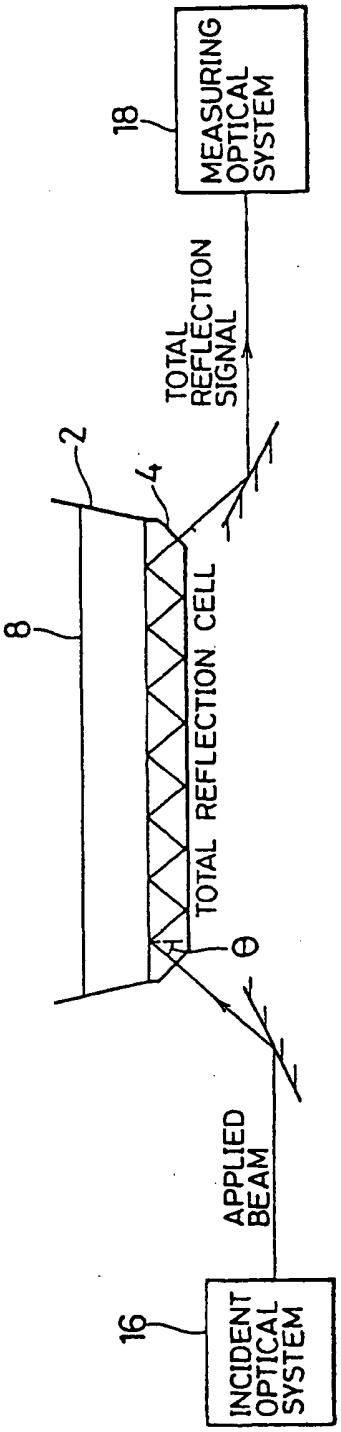

18
MEASURING OPTICAL SYSTEM
TOTAL REFLECTION SIGNAL
2
4
8
TOTAL REFLECTION CELL
θ
APPLIED BEAM
16
INCIDENT OPTICAL SYSTEM

Fig. 2

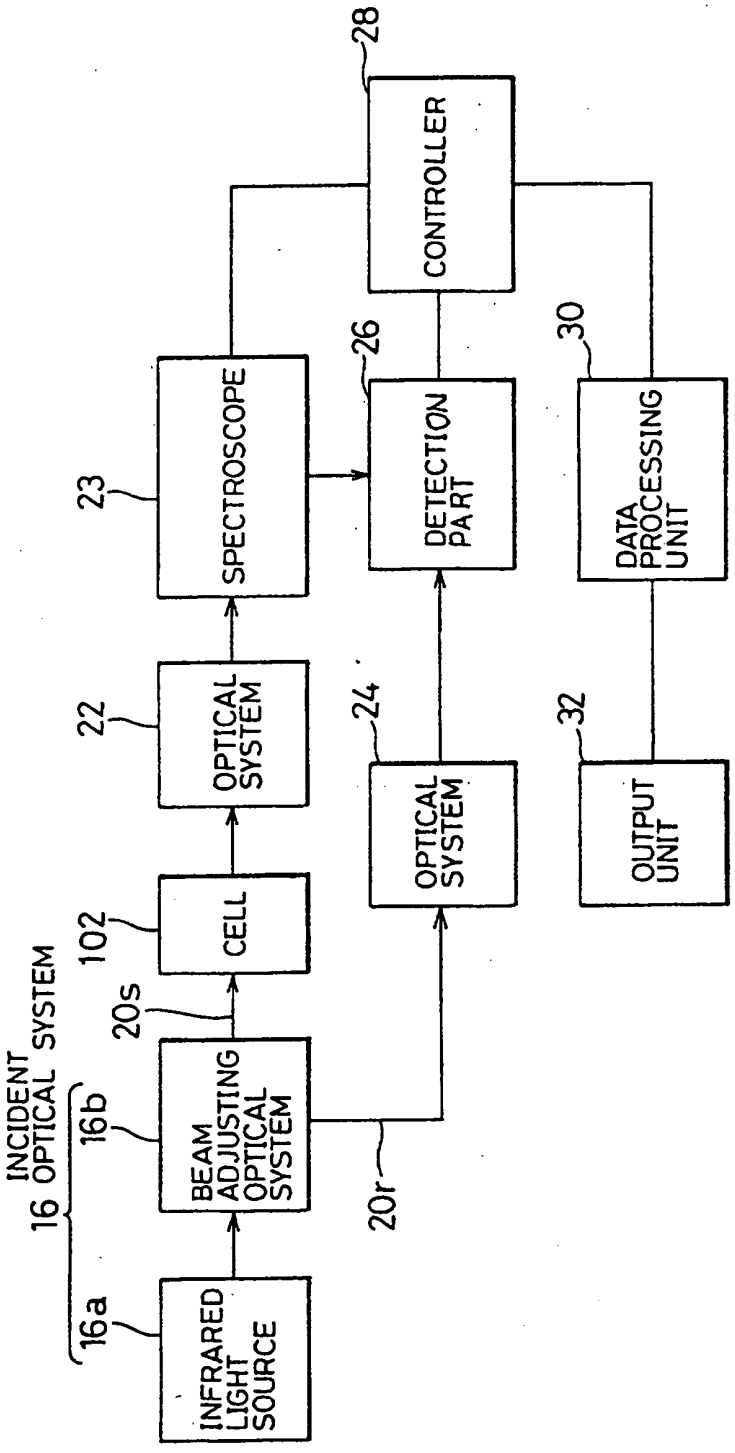

INFRARED LIGHT SOURCE
16a
INCIDENT OPTICAL SYSTEM 16
16b
BEAM ADJUSTING OPTICAL SYSTEM
20s
CELL
102
OPTICAL SYSTEM
22
SPECTROSCOPE
23
20r
OPTICAL SYSTEM
24
DETECTION PART
26
CONTROLLER
28
DATA PROCESSING UNIT
30
OUTPUT UNIT
32

Fig. 3A

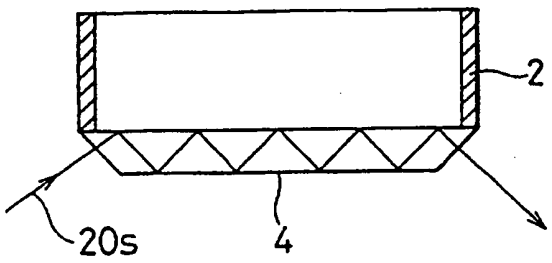


Fig. 3B

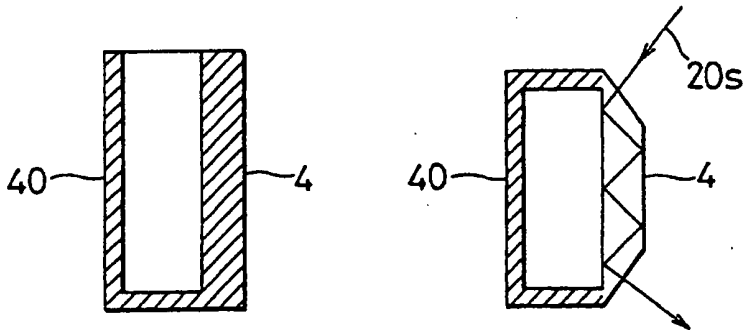


Fig. 4A

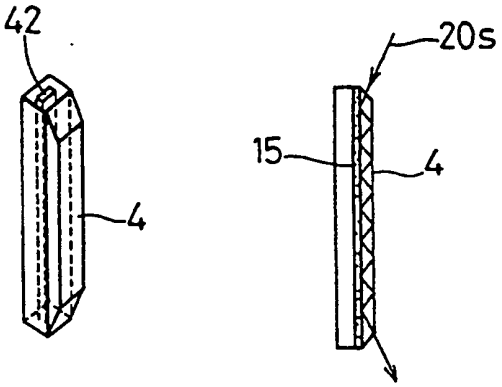


Fig. 4B

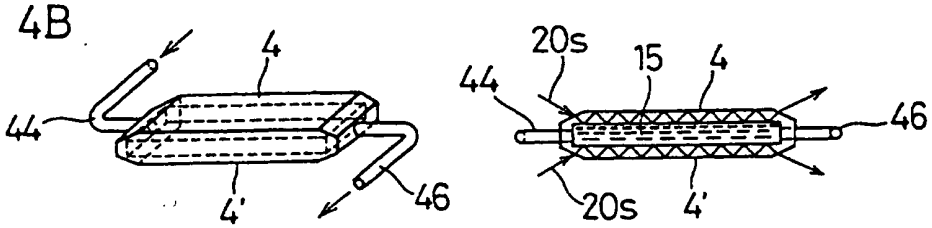


Fig. 4C

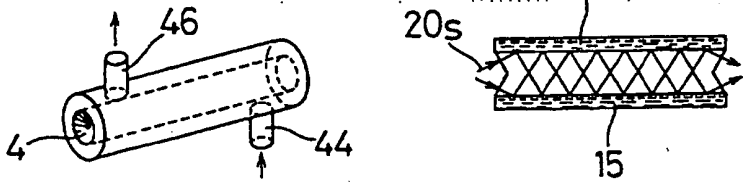


Fig. 4D

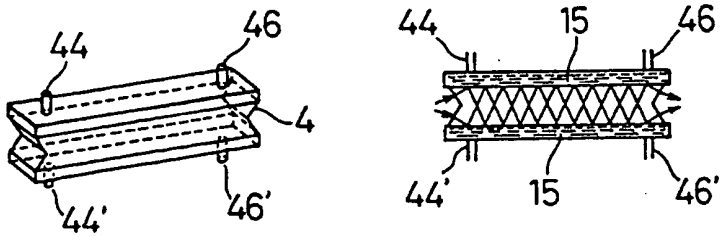


Fig. 4E

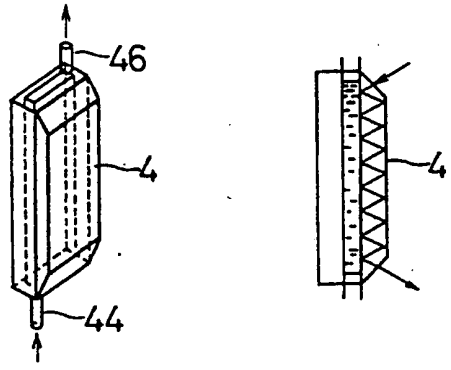


Fig. 5

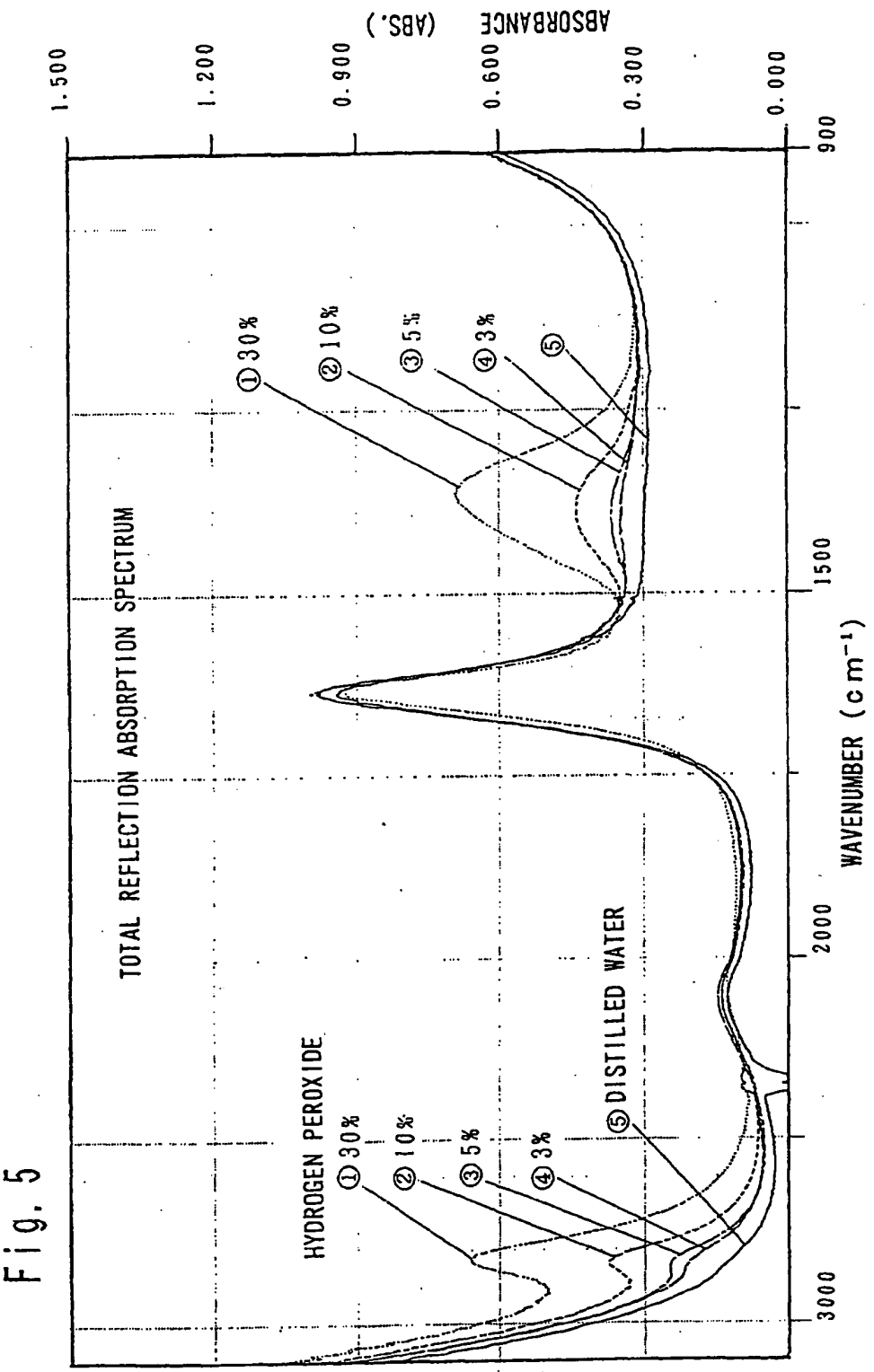

TOTAL REFLECTION ABSORPTION SPECTRUM
ABSORBANCE (ABS.)
1.500
1.200
0.900
0.600
0.300
0.000
900
1500
2000
3000
WAVENUMBER (cm⁻¹)
①30%
②10%
③5%
④3%
⑤
HYDROGEN PEROXIDE
①30%
②10%
③5%
④3%
⑤DISTILLED WATER

Fig. 6

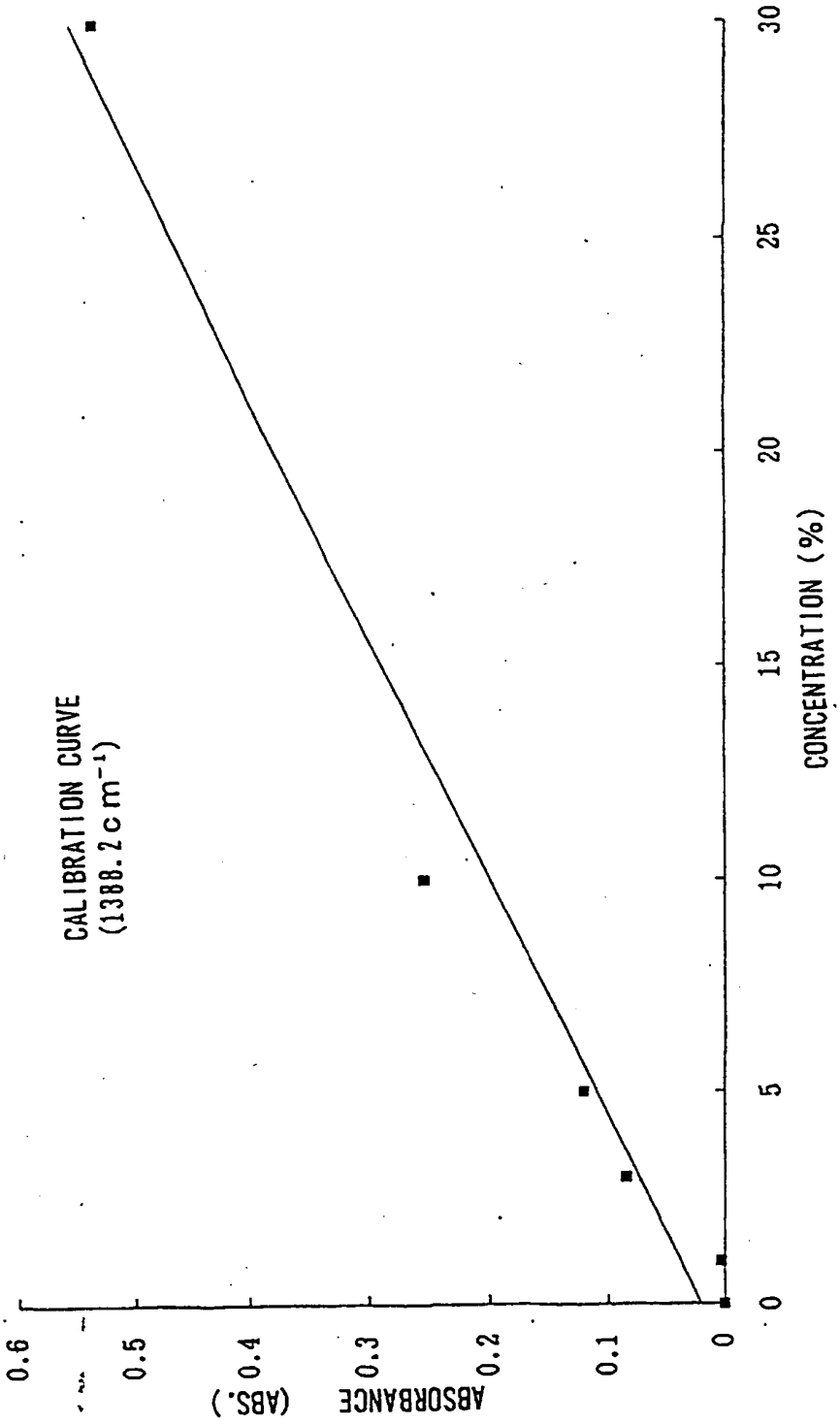

CALIBRATION CURVE
(1388.2cm⁻¹)
ABSORBANCE (ABS.)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 7

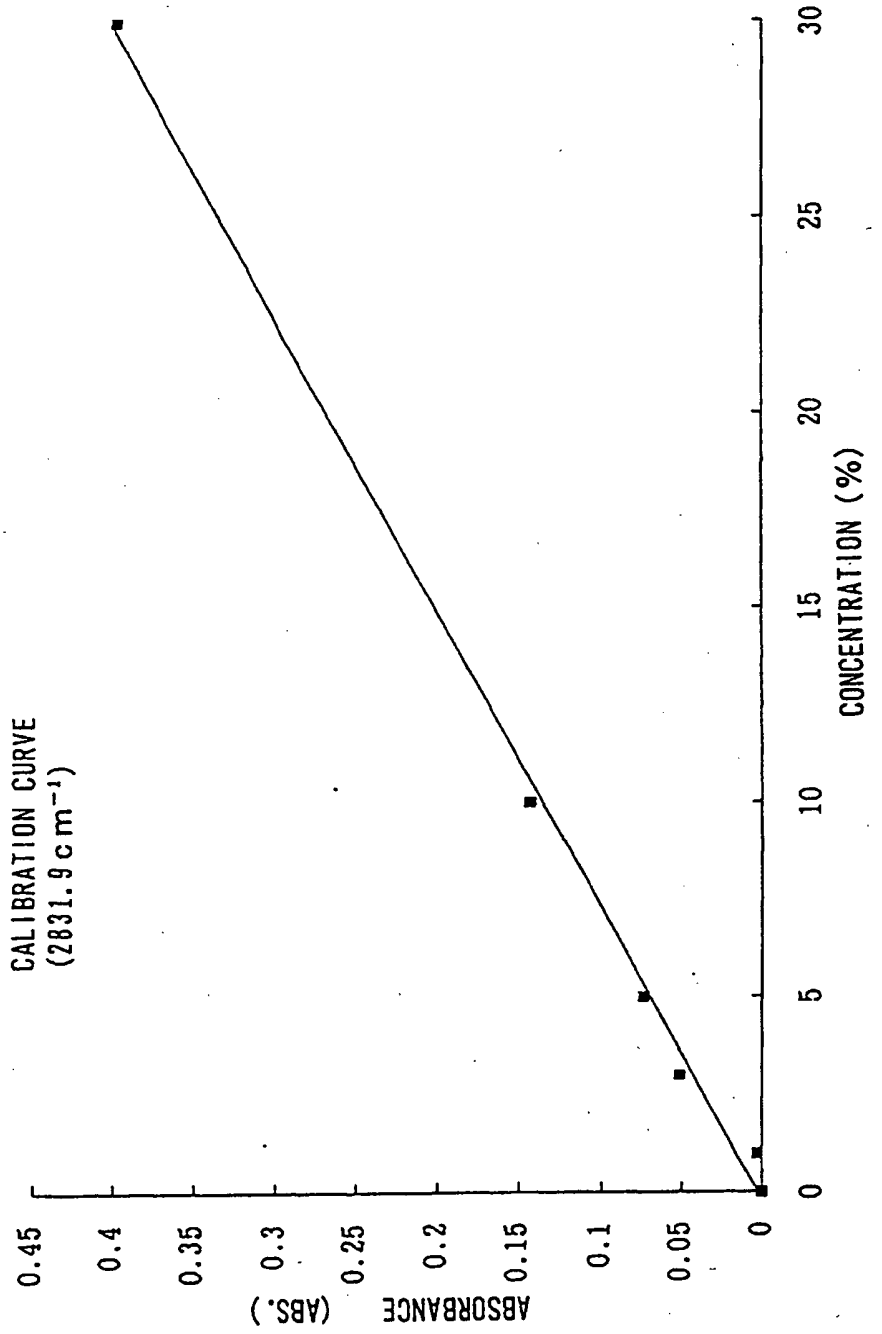

CALIBRATION CURVE
(2831.9cm⁻¹)
ABSORBANCE (ABS.)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 8

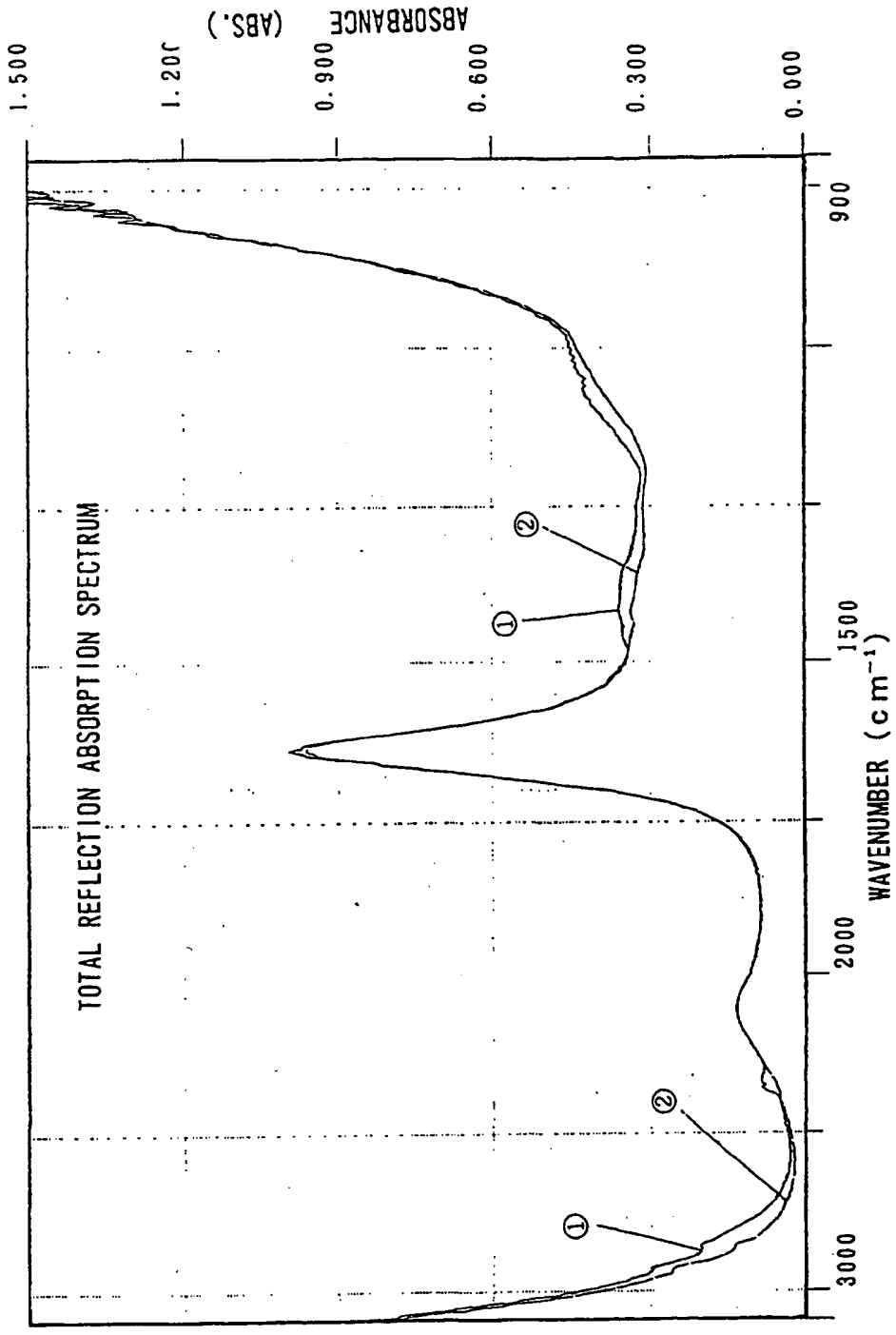

TOTAL REFLECTION ABSORPTION SPECTRUM
ABSORBANCE (ABS.)
1.500
1.200
0.900
0.600
0.300
0.000
900
1500
2000
3000
WAVENUMBER (cm⁻¹)
①
②
①
②

Fig. 9

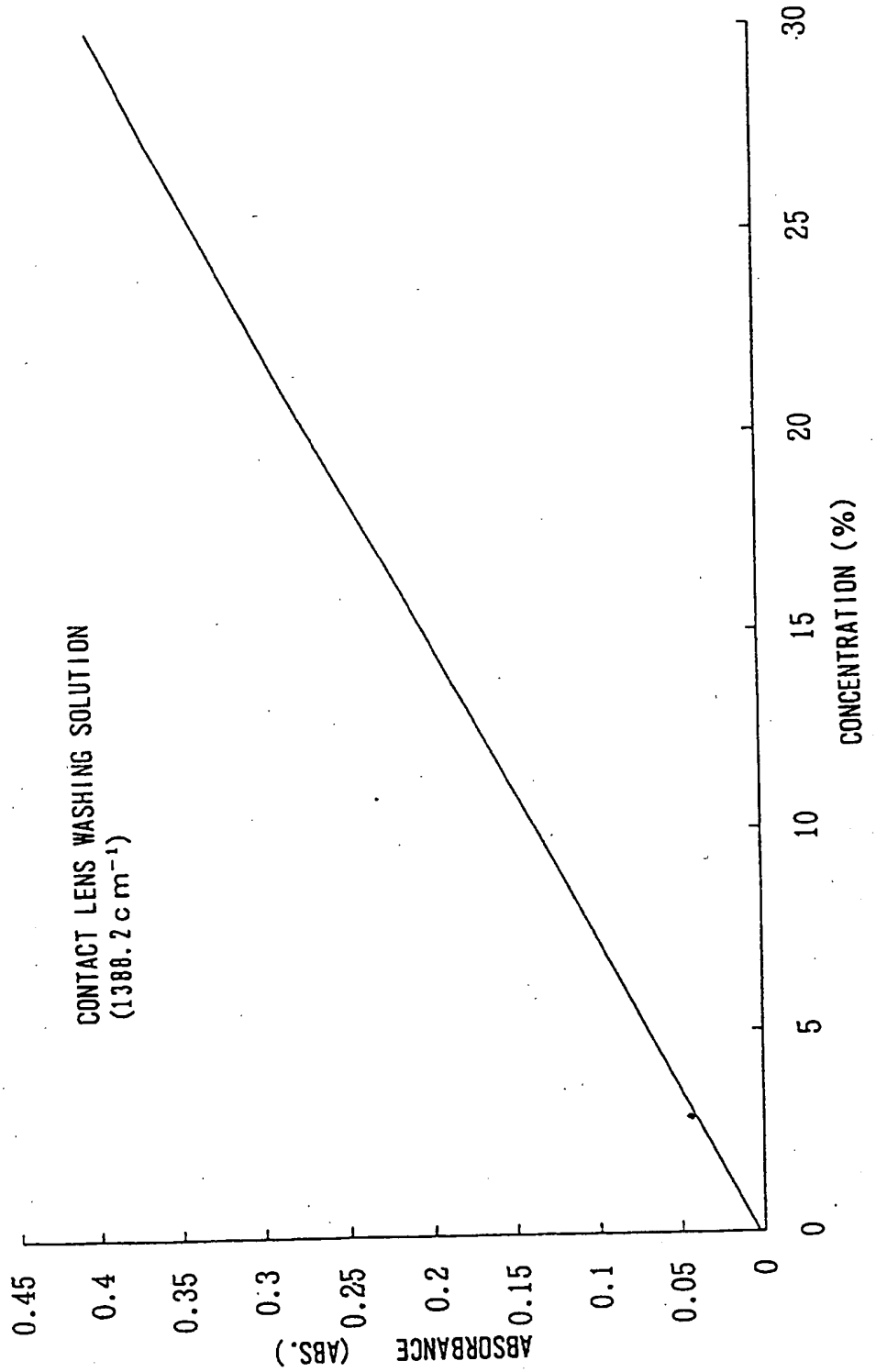

CONTACT LENS WASHING SOLUTION
(1388.2cm⁻¹)
ABSORBANCE (ABS.)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 10

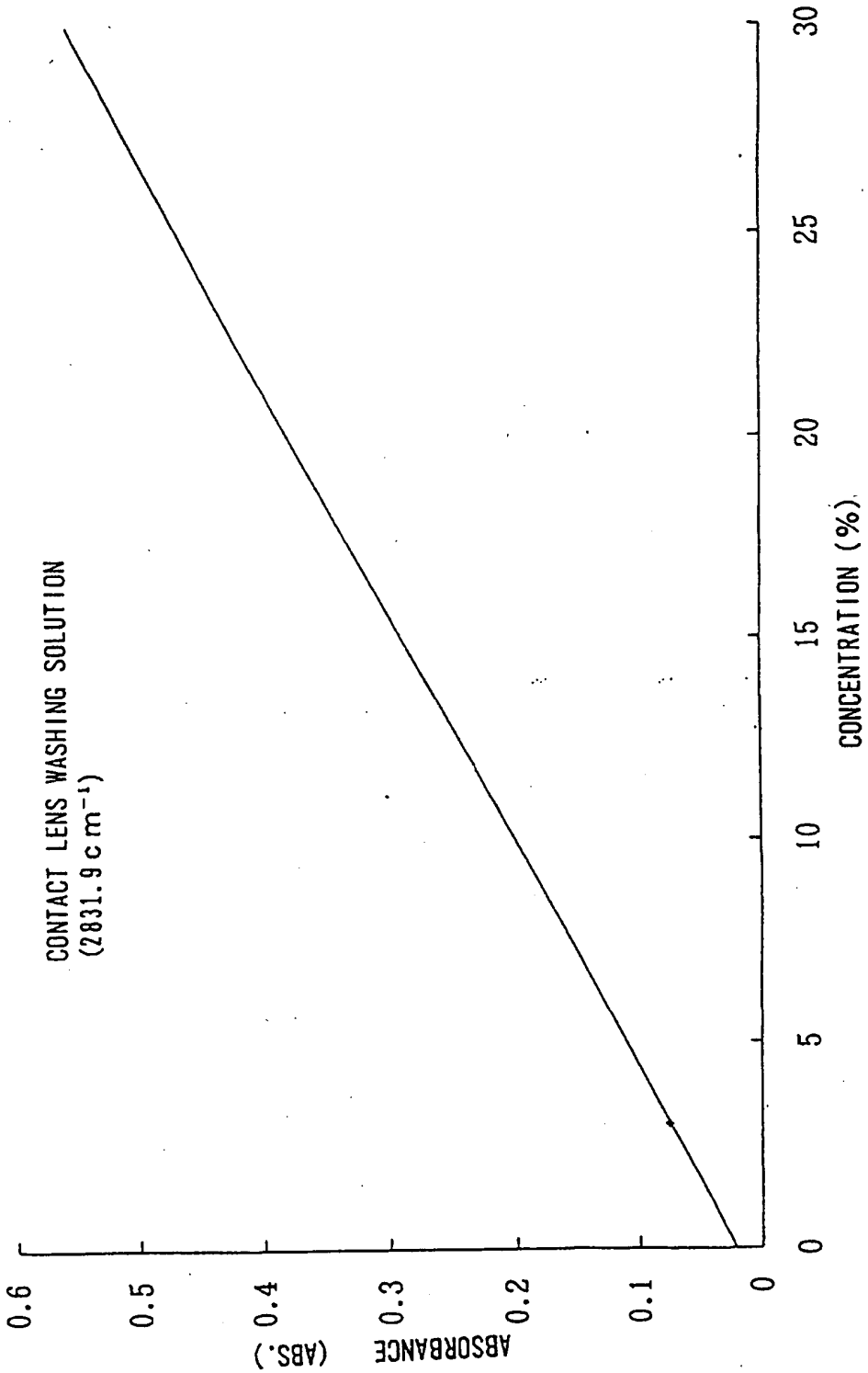

CONTACT LENS WASHING SOLUTION
(2831.9 c m⁻¹)
ABSORBANCE (ABS.)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 11

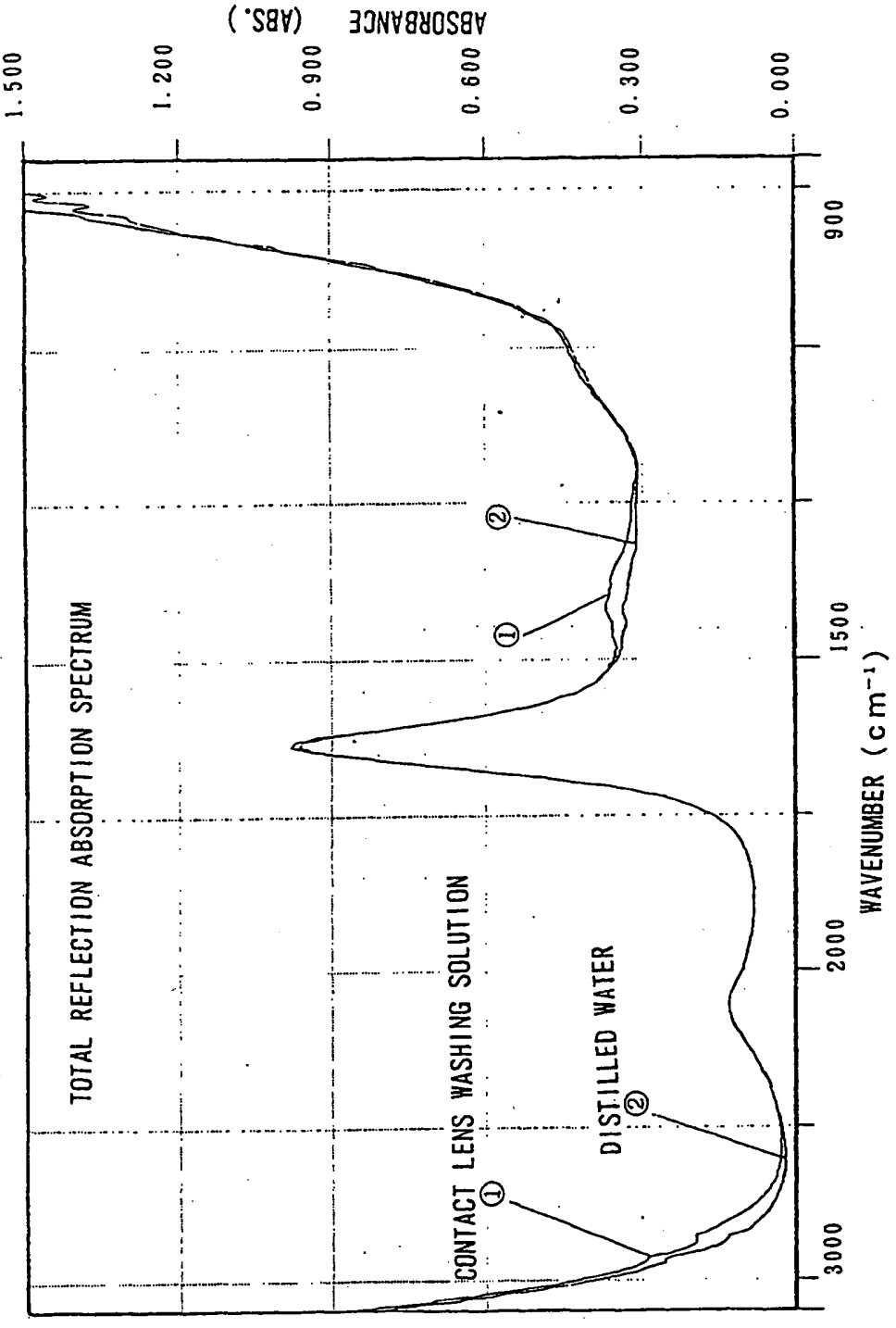

TOTAL REFLECTION ABSORPTION SPECTRUM
ABSORBANCE (ABS.)
1.500
1.200
0.900
0.600
0.300
0.000
900
1500
2000
3000
WAVENUMBER (cm⁻¹)
CONTACT LENS WASHING SOLUTION
①
DISTILLED WATER
②
②
①

Fig. 12

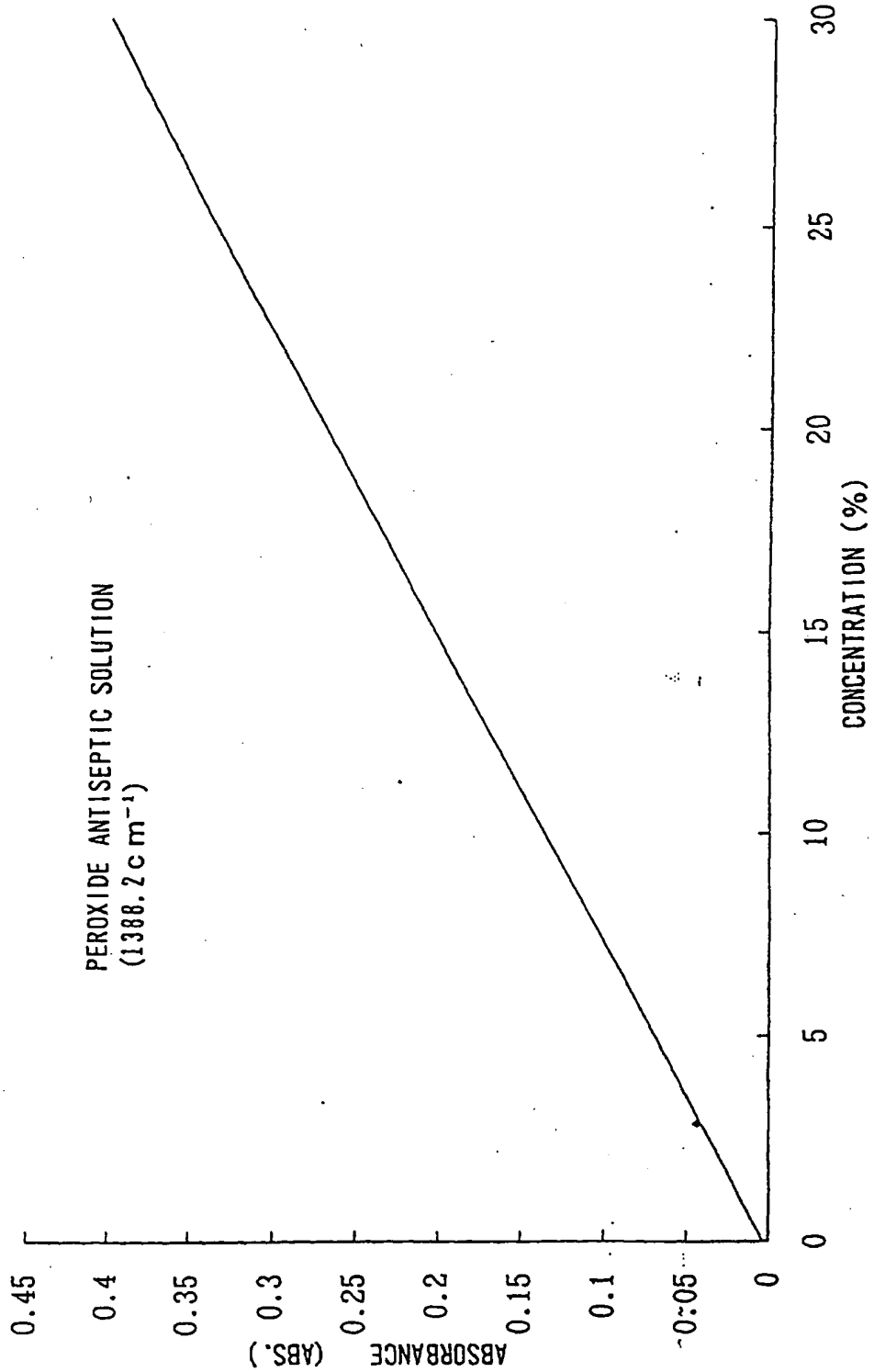

PEROXIDE ANTISEPTIC SOLUTION
(1388.2cm⁻¹)
ABSORBANCE (ABS.)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 13

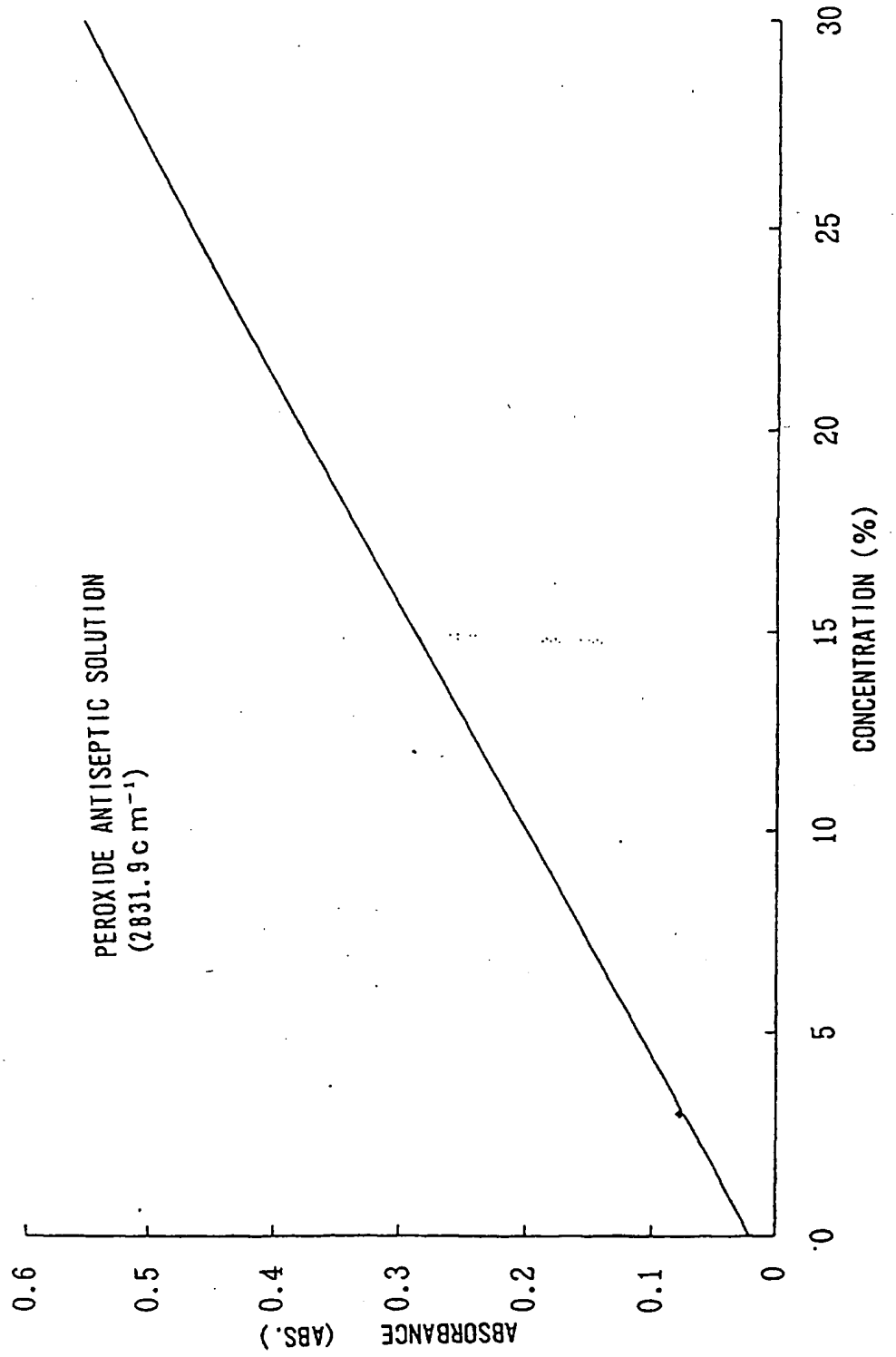

PEROXIDE ANTISEPTIC SOLUTION
(2831.9cm⁻¹)
ABSORBANCE (ABS.)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
CONCENTRATION (%)

Fig. 14

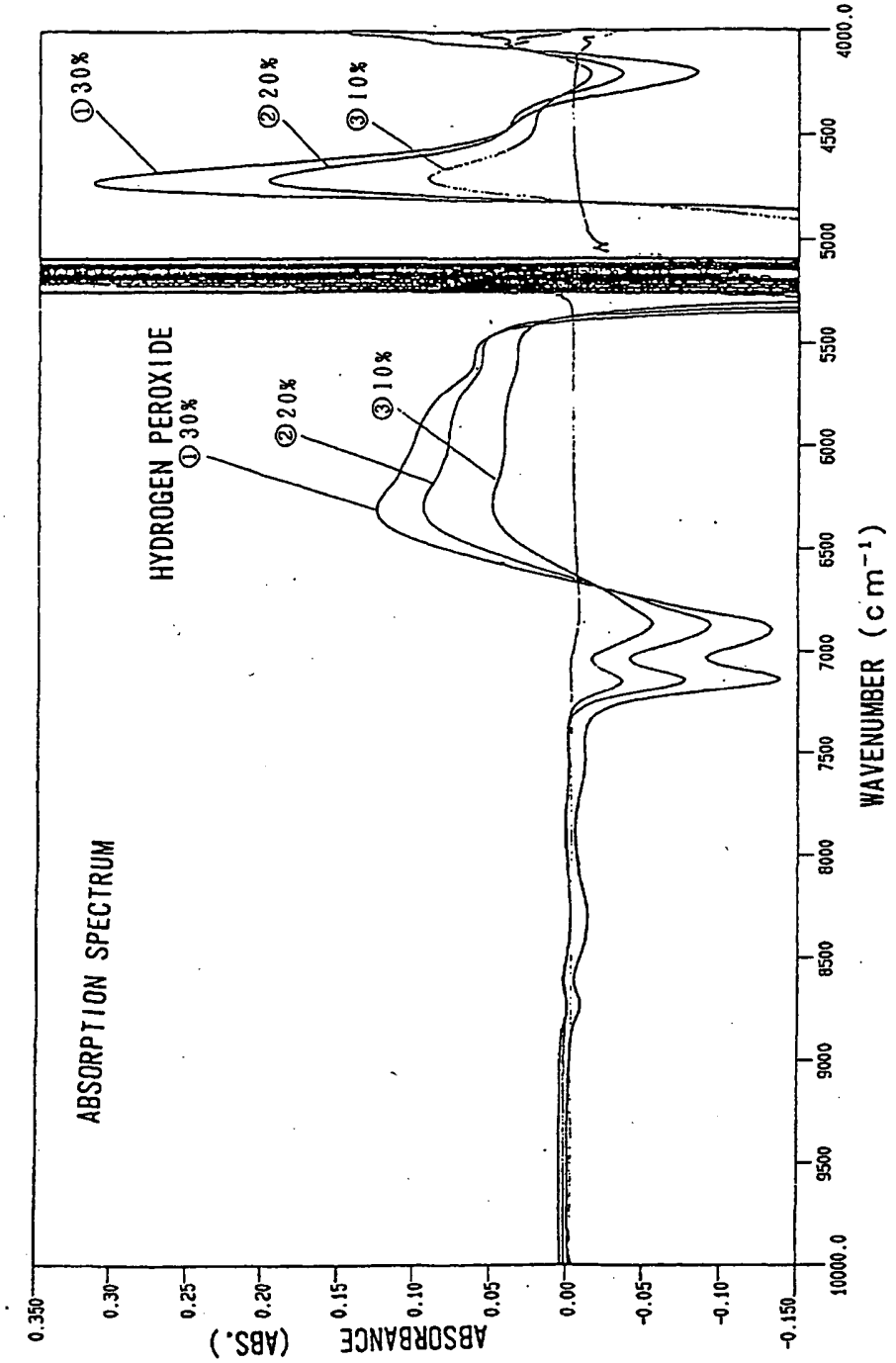

ABSORPTION SPECTRUM
HYDROGEN PEROXIDE
①30%
②20%
③10%
①30%
②20%
③10%
ABSORBANCE (ABS.)
WAVENUMBER (cm⁻¹)
0.350
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.150
10000.0
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000.0

Fig. 15

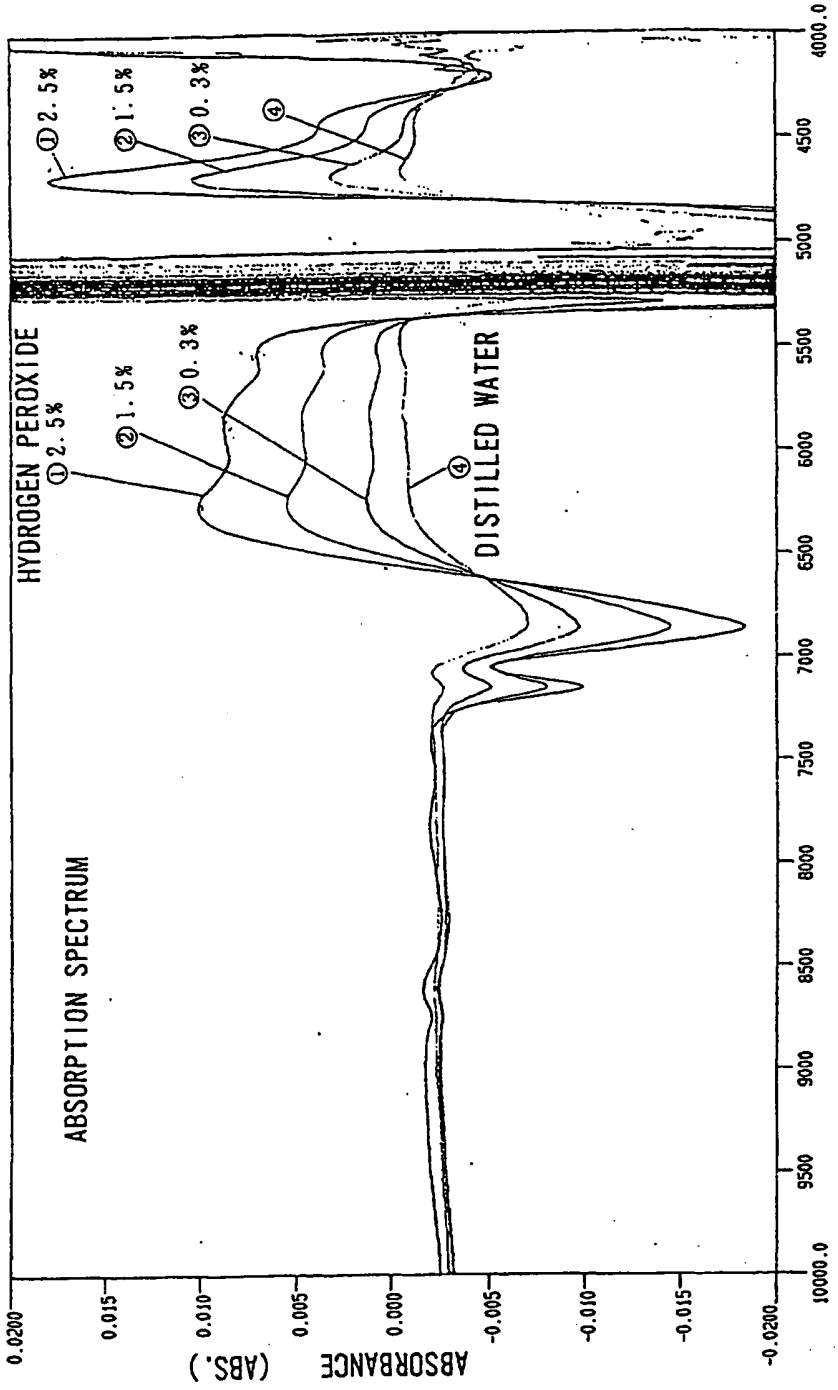

ABSORPTION SPECTRUM
HYDROGEN PEROXIDE
① 2.5%
② 1.5%
③ 0.3%
④ DISTILLED WATER
ABSORBANCE (ABS.)
0.0200
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.0200
4000.0
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000.0

Fig. 16

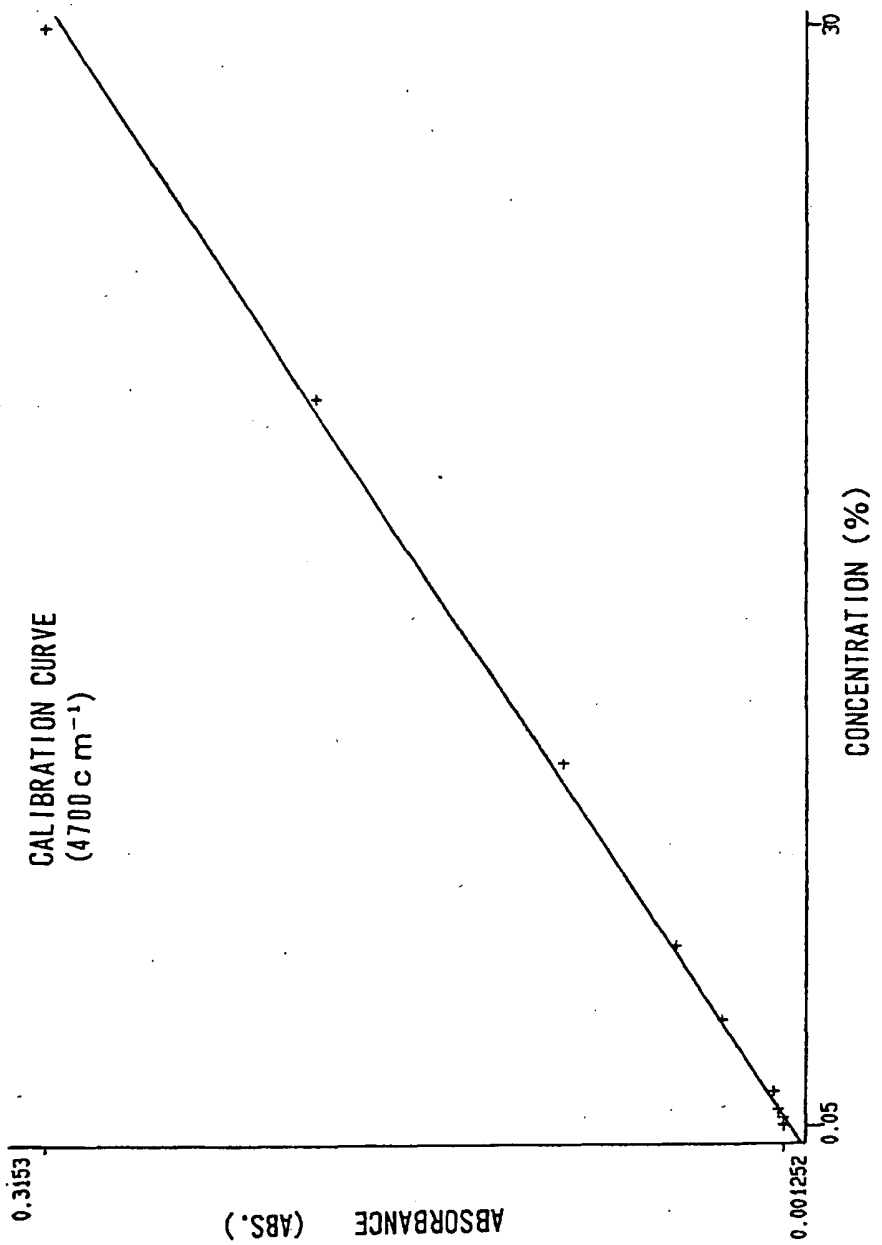

CALIBRATION CURVE
(4700cm⁻¹)
ABSORBANCE (ABS.)
0.3153
0.001252
0.05
30
CONCENTRATION (%)

Fig. 17

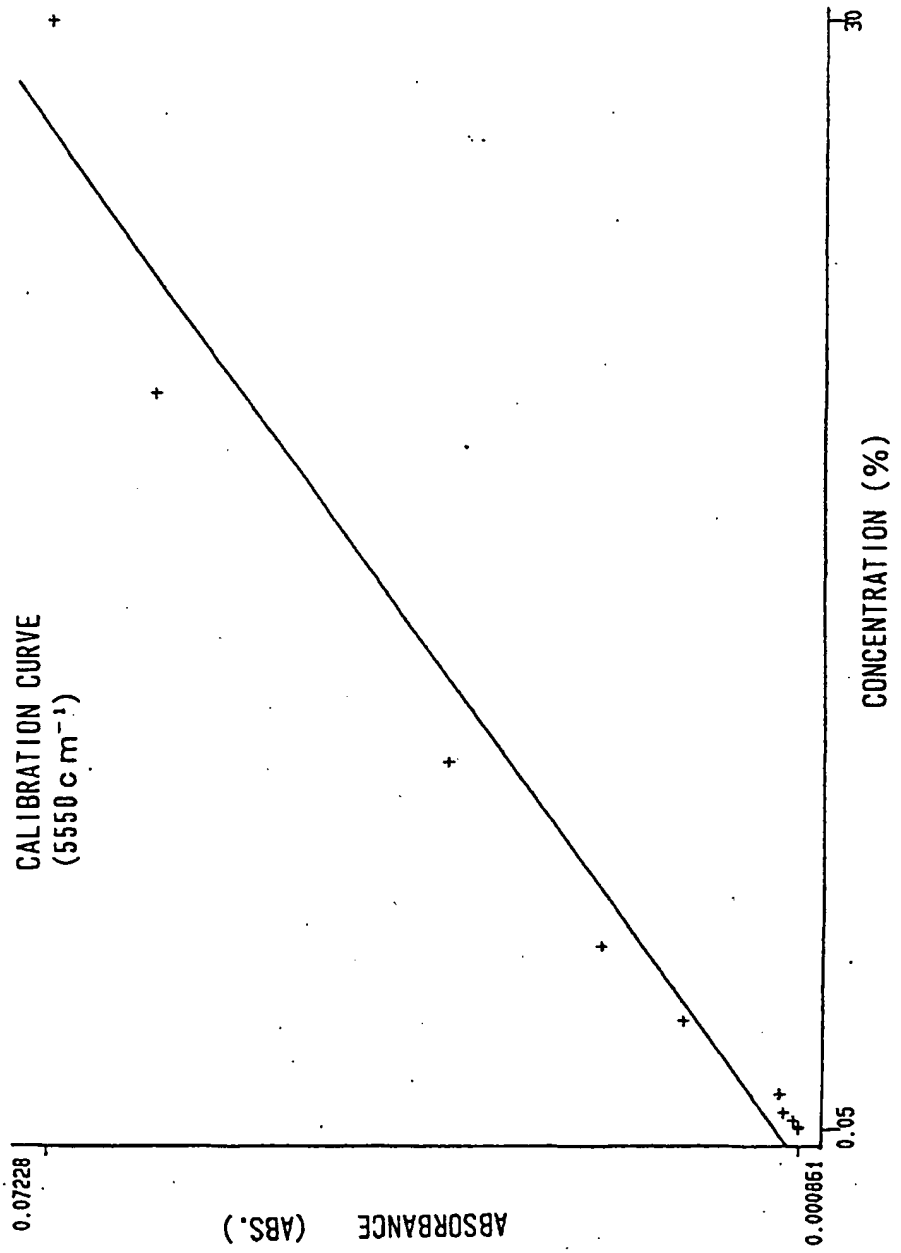

CALIBRATION CURVE
(5550cm⁻¹)
ABSORBANCE (ABS.)
0.07228
0.000861
0.05
30
CONCENTRATION (%)

Fig. 18

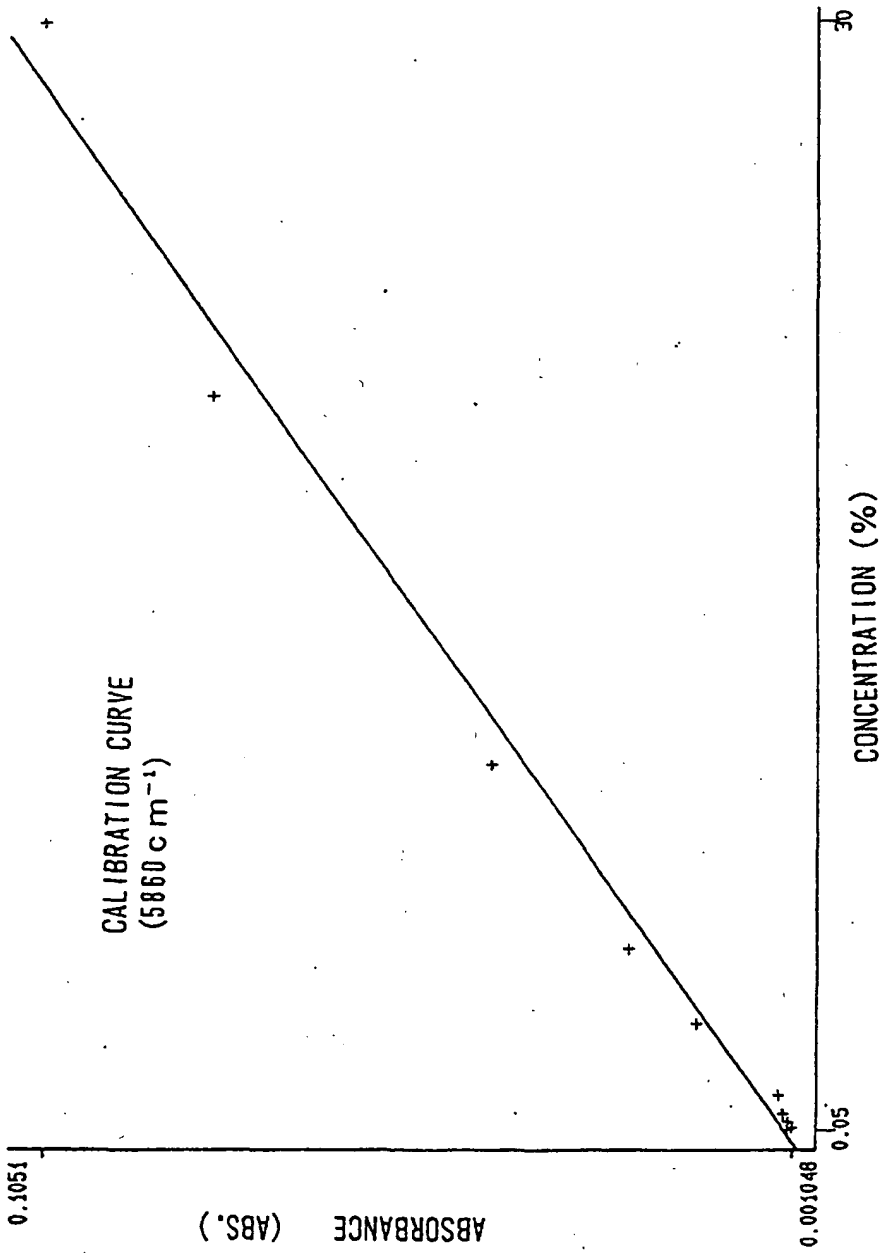

CALIBRATION CURVE
(5860 c m⁻¹)
ABSORBANCE (ABS.)
0.1051
0.001048
0.05
30
CONCENTRATION (%)

Fig. 19

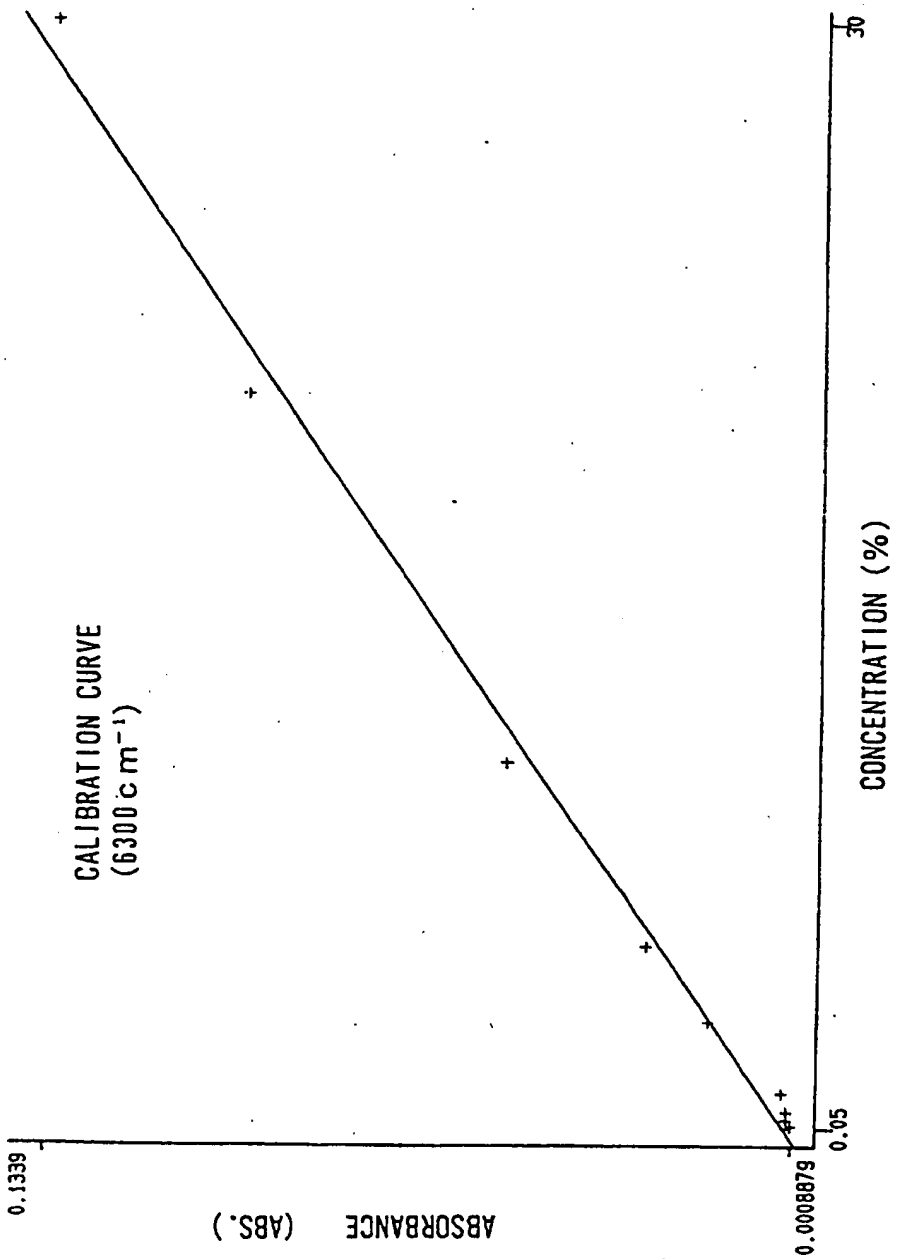

CALIBRATION CURVE
(6300 c m⁻¹)
ABSORBANCE (ABS.)
0.1339
0.0008879
0.05
30
CONCENTRATION (%)

Fig. 20

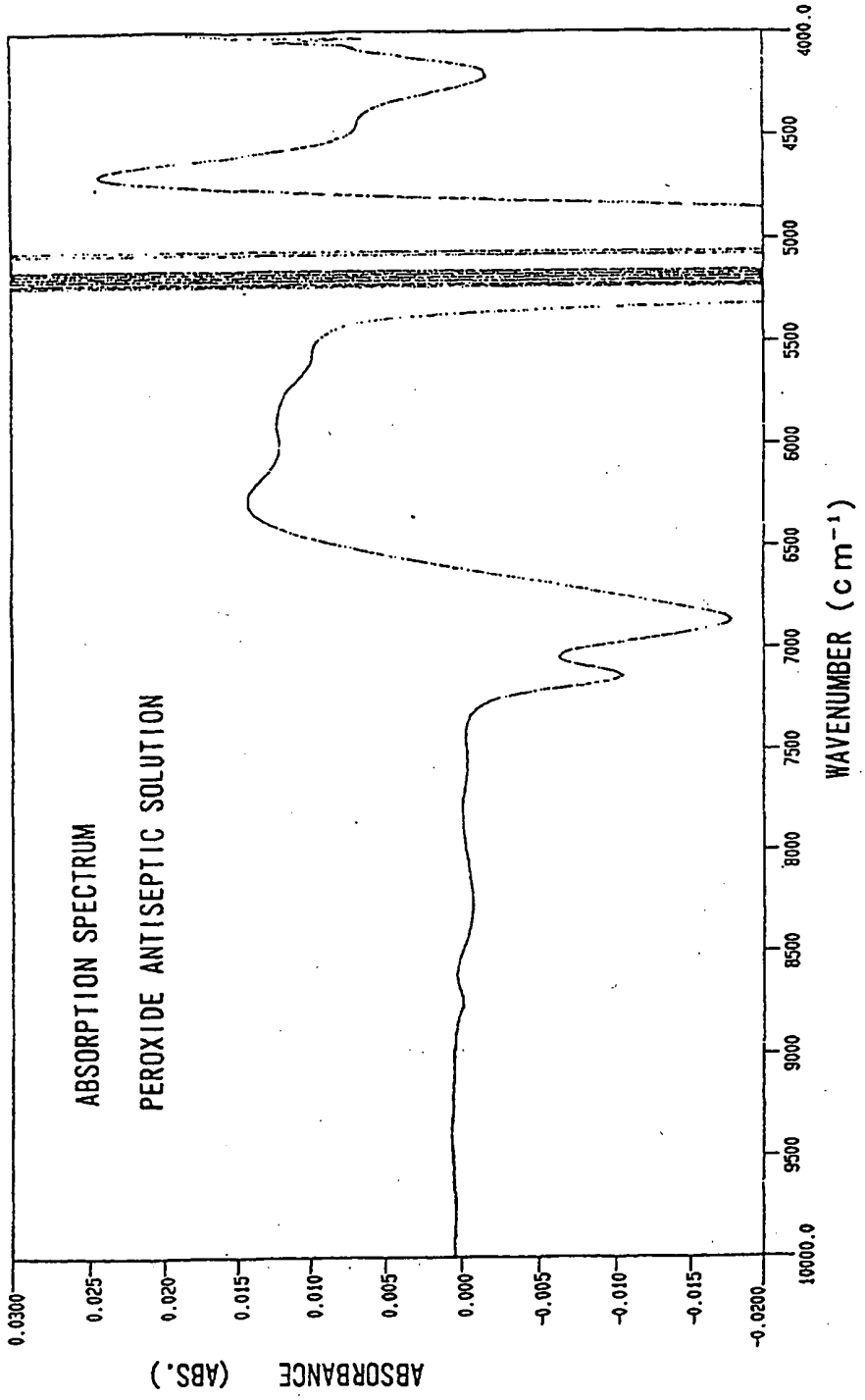
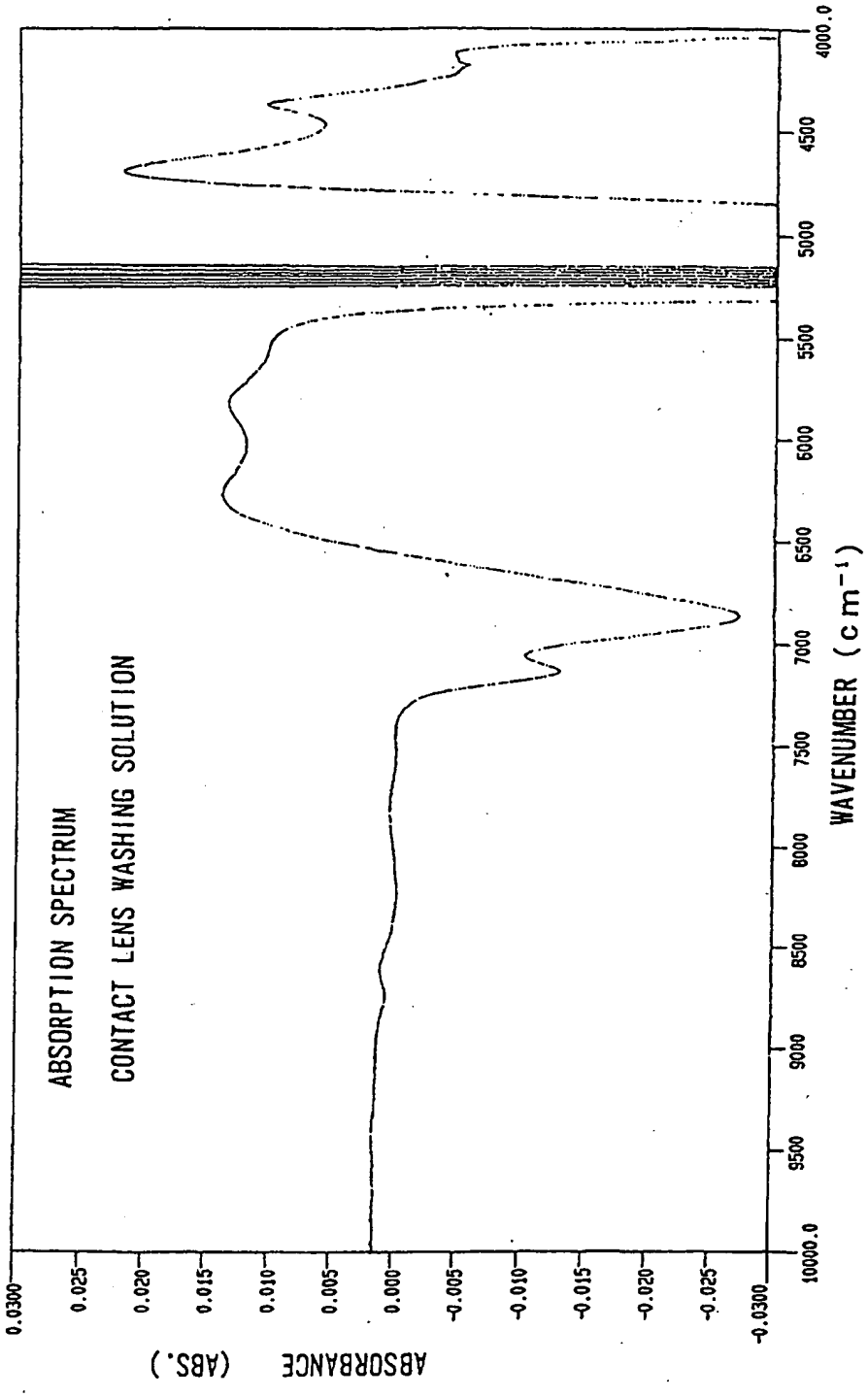

ABSORPTION SPECTRUM
PEROXIDE ANTISEPTIC SOLUTION
ABSORBANCE (ABS.)
0.0300
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.0200
4000.0
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000.0
WAVENUMBER (cm⁻¹)

Fig. 21


ABSORPTION SPECTRUM
CONTACT LENS WASHING SOLUTION
ABSORBANCE (ABS.)
0.0300
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.0300
WAVENUMBER (cm⁻¹)
4000.0
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000.0